LEPIDOPTERAN EGG PARASITOID SURVEY OF O‘AHU: ASSESSING PARASITISM RATES AND PARASITOID DIVERSITY IN WILD COLLECTED AND SENTINEL EGGS ACROSS AN ELEVATION GRADIENT

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Chapter 1. An Overview of Native and Introduced Egg Parasitoids in Island Systems

Abstract:

Egg parasitoids are mostly wasps and flies that oviposit into the eggs of other insects, which are used as a food source for the development of their larvae. They are often purposely employed to control pests in both agricultural and natural settings through the resulting reduction in the populations of targeted pest insects. Small oceanic island systems are unique due to their isolation. This remoteness and separation can result in the evolution of native faunas unlike those of continents. Although islands are particularly prone to invasion by introduced species, currently there is no review summarizing the history of egg parasitoid introductions, together with native species checklists from archipelagos across the world. The purpose of this literature review is to foster future research by summarizing the history of egg parasitoid introductions across smaller, isolated oceanic islands. This review focuses on the introduction and discovery of egg parasitoids in island systems no greater than 16,637 km2. The egg parasitoid communities of the following archipelagos are summarized: Azores, Canary Islands, Galapagos, French Polynesia, Hawai‘i, and Micronesia.

Introduction:

Owing to their high levels of faunal and floral endemism in island ecosystems, which represent a significant portion of Earth’s biota, they are of great scientific interest and conservation concern (Kier et al. 2009). They are especially prone to the establishment of nonnative and potentially invasive species that are either accidently or intentionally introduced

by humans (Messing and Wright 2006; Peck et al. 1998; Funasaki et al. 1988). The establishment of such invasive species often results in significant negative impacts on island ecosystems, which can eventually lead to the extinction of endemic species (Howarth 1991). These impacts include both habitat alteration and negative interactions with native species, including competition, predation, and hybridization (Elkinton et al. 2010; Havill et al. 2012; Yara et al. 2010). Invasive species can also retard socioeconomic growth when they are harmful to important economic resources such as crops or environmental services (Dobson 1988; Reaser et al. 2007). Because of these potential impacts, classical biological control (CBC) is employed to suppress pest species, especially in island systems, where native predators or parasitoids may be absent (Reaser et al. 2007). Of the four main types of biological control — classical biological control, inoculative augmentative biological control, inundative augmentative biological control, and conservation biological control (Eilenberg et al. 2001) — classical biological control is the primary method for control of foreign pests for two major reasons: it is cost effective, and considered an environmentally safe option due to a decreased need for chemical pesticides (Van Lenteren et al. 2003). However, this method of biological control was not used globally until the start of the 19th century, when parasitoids and other predators were employed as biocontrol in Europe, and later in the United States, against adventive crop pests (Bosch et al. 1982). Currently, the use of egg parasitoids to control pests is quite common, with many documented cases in both conservation and agricultural contexts (Roriz et al. 2006; Smith 1996; Hassan 1993; Clarke 1990).

Egg parasitoids are a group of parasitoids that oviposit their eggs into the eggs of other insects; they are usually wasps, although some flies are egg parasitoids. The most notable egg parasitoids belong to the following Hymenoptera families: Trichogrammatidae, Mymaridae, Encyrtidae, and Platygastridae. Examining the history of biocontrol using egg parasitoids across

different archipelagos can reveal those species which have been successful. Such information is valuable for predicting the parasitoids present on archipelagos yet to be surveyed, and in helping future biological control projects by highlighting which species and genera have been previously successful in controlling pests. This chapter aims to summarize the historical use and discoveries of egg parasitoids in several islands and archipelagos across the world, the taxonomy of each parasitoid, and whether they were purposefully introduced for biological control. Through study of the history of egg parasitoids used in biological control we may prevent the introduction of egg parasitoids that may not only be ineffective, but also potentially harmful to the native fauna. This review provides a brief summary of the history of established introductions and significant discoveries of egg parasitoids in several archipelagos, each with a land area no greater than 16,636 km2. The land area was decided from the size of the Hawaiian Islands which is the largest archipelago for its remote location. The egg parasitoid communities of the Azores, Canary Islands, French Polynesia, Galapagos, Hawaiian Islands, and Micronesia are summarized.

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A Geographical Survey of Egg Parasitoids

Azores:

The Azores are a Portuguese volcanic archipelago of nine islands encompassing a land area of 2,347 km2 in the mid-Atlantic Ocean. In total there have been two documented egg parasitoid surveys and a total of ten species of egg parasitoids recorded (Table 1.1).

In 1989 a parasitoid survey was conducted on São Miguel island which identified four species of egg parasitoids belonging to three families of Hymenoptera (Table 1.1). These wasps

were only collected from four out of six surveyed localities, and trichogrammatids were only found in locations at low elevation (Pintureau et al. 1990).

Another egg parasitoid survey of São Miguel Island was conducted between November 1991 and November 1993, through weekly surveys of wild lepidopteran eggs belonging to the following species: *Autographa gamma* L. (Noctuidae), *Peridroma saucia* Hübner (Noctuidae), *Chrysodeixis chalcites* Esper (Noctuidae), and *Udea ferrugalis* Hübner (Crambidae). Only two wasps: *Telenomus* sp. (Scelionidae), and *Trichogramma cordubensis* Vargas & Cabello (Trichogrammatidae) were retrieved following the rearing of 21,946 eggs, which had been collected from six species of plants. The total number of plants surveyed was 1,200 (200 per species), and both of the identified egg parasitoid wasps were found to be polyphagous on multiple species of Lepidoptera (Garcia et al. 1995b).

A study conducted between 1991-1992 found that different populations of *T. cordubensis* showed different capacities for the quantity of eggs they could parasitize, but parasitism always peaked within the first three days of the wasps' emergence (Garcia et al. 1995a). The populations of *T. cordubensis* were found to have come from three different islands in the Azores: São Miguel, São Jorge, and Pico, (Garcia et al. 1995a). To determine whether the polyphagous *T. cordubensis* exhibited any host preference, a test of host suitability and preference was conducted, but no preferences were found (Garcia et al. 1995a). However, Roriz et al. (2006) did find that Lepidoptera with larger/heavier eggs experienced higher parasitism rates compared to hosts with smaller eggs, not only in how often they were targeted, but also in how many wasps emerged per egg (Roriz et al. 2006).

In 2006 a trichogrammatid new to the Azores was found on São Miguel. This species, *Trichogramma achaeae* Nagaraja & Nagarkatti, is distributed worldwide and is known to attack

27 species of Lepidoptera (Cabello et al. 2009; Pacilly et al. 2011). Recently, both *T. cordubensis* and *T. achaeae* have been tested as biological control agents against the introduced tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae) on São Miguel. Although *T. achaeae* was found to be a better control agent than *T. cordubensis*, both exhibited low emergence rates (Oliveira et al. 2017). There is an online database titled "Base de Dados da Biodiversidade dos Açores" (BIOTA Azores (2019)) which includes egg parasitoids from the Azores (Table 1.1).

Canary Islands:

The Canary Islands are a Spanish volcanic archipelago comprised of seven main islands situated to the north-west of Africa: Tenerife, Fuerteventura, Gran Canaria, Lanzarote, La Palma, La Gomera, and El Hierro. The total area of this archipelago is 7,493 km2 There have been two known surveys of egg parasitoids within this archipelago, with 22 known egg parasitoid species documented (Table 1.2).

In 2004 a list of known Hymenoptera species including egg parasitoids from the Canary Islands was published (Baez et al. 2004). A different list was compiled in 2004 for the Eupelmidae of the Iberian Peninsula and the Canary Islands with one additional egg parasitoid found from the Canary Islands (Table 1.2) (Askew & Nieves 2004).

In 2008 a survey was conducted which reported *Trichogramma* for the first time on this archipelago. This study surveyed eggs of *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) and *Chrysodeixis chalcites* Esper (Lepidoptera: Noctuidae) on the following islands: Tenerife, La Palma, El Hierro, and Gran Canaria (Polaszek et al. 2012). The second survey was conducted from 2008 to 2009, which collected and sequenced *Trichogramma* species from *C. chalcites*

eggs in the Canary Islands. The following islands and their respective species were catalogued: Tenerife (*T. achaeae*, *T. bourarachae*, Pintureau & Babault, *T. euproctidis*, Girault, and *T. evanescens,* Westwood), La Palma (*T. achaeae*), Gran Canaria (*T. achaeae*, and *T. nr. brassicae*), and El Hierro (*T. achaeae*). Polaszek et al. (2012) investigated the density of each *Trichogramma* species in banana crops on the islands, revealing *T. euproctidis* to be the dominant wasp in banana plantations on Tenerife, *T. achaeae* to be dominant on La Palma, and both *T. achaeae* and *T.* sp. nr. *brassicae* to occur in approximately equal numbers on Gran-Canaria.

In 2014 a catalogue of new records of Mymaridae in the Canary Islands and Madeira was published with eight adventive species from the Canary Islands (Table 1.2) (Koponen & Trjapitzin 2014).

None of the *Trichogramma* species previously found in the Canary Islands were thought to be native to those islands, as they were previously recorded from Asia, mainland Europe, and Africa (Del Pino et al. 2013). However, in 2012 the previously recorded trichogrammatid, *T. sp.* nr. *brassicae,* was described as a new species, *T. canariensis* Del Pino, and is now considered an endemic species only known from this island (Del Pino et al. 2013).

French Polynesia:

French Polynesia includes the following archipelagos: The Society Islands, Austral Islands, Marquesas, and the Tuamotu Archipelago. These archipelagoes are highly isolated in the South Pacific Ocean and cover a total land area of 4,167 km2. There are a total of 11 species of egg parasitoids in the French Polynesian islands including two endemic species (Table 1.3).

In 1928, Cheesman catalogued the insect fauna previously collected in French Polynesia. In her records were two egg parasitoids from the Family Evaniidae (*Evania appendigaster*, and *E. sericea*) which had been documented in 1758 and 1883 and then redocumented in Cheesman's latest survey in the Marquesas Islands (Cheesman 1928).

In 1999 the glassy-winged sharpshooter *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) invaded Tahiti (Grandgirard et al. 2008), but biological control was not implemented until 2005 with the introduction of *Gonatocerini ashmeadi* (Hymenoptera: Mymaridae). Its rate of parasitism on *H. vitripennis* was around 90%, which encouraged its further introduction across French Polynesia (Petit et al. 2008).

I was unable to find more recent parasitoid surveys in the region. However there is a spreadsheet available online from Ramage (2017) which holds a great number of arthropod species documented over the years from French Polynesia (Ramage 2017).

Galapagos Islands

The Galapagos Islands is a volcanic archipelago in the Pacific Ocean and has a total land area of 7,880 km2. This archipelago is known for its pristine and protected wildlife with over 95% of the islands being protected in the Galapagos National Park.

In 1998 a study was done to look at the introduced insect fauna within the Galapagos Islands. This research was a culmination of past literature from previous entomologists, previously published field studies, and the 10-year sampling from the authors of this study (Peck et al. 1998). In this study a total of five egg parasitoids were documented to have been accidentally introduced into the Galapagos (Table 1.4) (Peck et al. 1998). No other articles regarding egg parasitoids within the Galapagos were found, but there is an online database that has all records of species known to the islands. No additional egg parasitoid records were found in this database (CDF 2019).

Hawaiian Islands

The Hawaiian Islands are an isolated volcanic archipelago in the north central Pacific, consisting of seven larger islands: Hawai‘i, Maui, Lāna‘i, Moloka‘i, O‘ahu, Ni‘ihau and Kaua‘i and a long series of small islets and atolls extending to the northwest of the main islands. The total land area of the archipelago is 16,637 km2. The Hawaiian Islands are renowned for their proportion of endemic species with over 5,000 described native insects alone, of which most are endemic (Nishida 2002). The islands are both a popular tourist destination and a crossroad for international trade in the Pacific, in addition to hosting a strong military presence. These three

qualities have contributed to the establishment of a multitude of introduced species across many taxa (Messing and Wright 2006). The islands are also a hotbed for biological control introductions and research. In the past century alone over 700 parasitoids, predators, and herbivores have been introduced to control invertebrate pests (Johnson et al. 2005). Below is a brief history of some significant introductions and discoveries of egg parasitoids in Hawai‘i. In total there have been at least 146 recorded species of egg parasitoids in the Hawaiian Islands, with over half of the species being confirmed non-native (114). Of these non-native species however, only 25 were purposefully introduced as biocontrol agents, while the remainder (89) are thought to be adventive (Table 1.5) (Nishida 2002).

In 1900 *Perkinsiella saccharicida*, Kirkaldy (Homoptera: Cicadellidae) became established in Hawai‘i and spread throughout the islands causing severe damage to sugarcane plantations. Three egg parasitoid species in the family Mymaridae were introduced to combat it: *Anagrus optabilis* Perkins, *A. perforator* (Perkins) and *A. frequens* Perkins (Triapitsyn and Beardsley 2000). The use of *Anagrus* species to suppress *P. sachharia* was successful (Triapitsyn and Beardsley 2000), and between 1915 and 1945 two more species of Mymaridae were introduced to control planthopper pests: *A. yawi* (from Mexico), and *A. columbi* (from Ohio).

In 1901 Ashmead compiled a catalogue of the parasitoids in Hawai‘i, listing five families of egg parasitoids (Appendix). Swezey (1929) produced notes on the egg parasitoids known from the islands, reviewing both native and introduced species in Hawai‘i (Appendix) (Swezey 1929). One subfamily of Braconidae present in Hawaii (Cheloninae) is known to attack eggs, in addition to larvae and the following egg parasitoid in the subfamily Cheloninae has been recorded: *Chelonus blackburni* Cameron (Midway, Pearl and Hermes, Laysan, Ni‘ihau, and all

the high Hawaiian Islands).

In 1904 Perkins and Koebele introduced the egg parasitoid *Aphanomerus pusillus* Perkins (Hymenoptera: Platygastridae) for the control of a leafhopper known as the torpedo bug, *Siphanta acuta* Walker (Hemiptera: Flatidae). This egg parasitoid was very successful in controlling the populations of *S. acuta*, which was then only seen in small numbers (Pemberton 1964a).

Between 1905 and 1916 two egg parasitoids: *Ootetrastichus beatus* Perkins and *O. formosanus* Timberlake (Hymenoptera: Eulophidae) were introduced for the control of a sugarcane leafhopper: *P. saccharicida*. The control from this wasp was generally successful except for one area on the island of Hawai‘i.

In 1928 a new egg parasitoid, *Anaphes calendrae* Gahan (Hymenoptera: Mymaridae), was introduced into Hawai‘i for the control of the New Guinea sugarcane weevil: *Rhabdoscelus obscurus* Boisduval (Coleoptera: Curculionidae). The establishment of this egg parasitoid was successful, but there is a lack of evidence that this parasitoid has been effective in the field (Beardsley 2000).

In 1946, the oriental fruit fly, *Bactrocera dorsalis* Hendel (Tephritidae) was detected in the Hawaiian Islands, and an introduced egg-larval parasitoid *Biosteres oophilus* now known as *B. arisanus* Sonan (Hymenoptera: Braconidae) was established from Mexico between 1947 and 1952 to suppress it, attaining up to 80% parasitization of its target pest (Wong et al. 1984)

Between 1961 and 1963 an egg parasitoid, *Telenomus (Trissolcus) basalis* (Wallaston) (Hymenoptera: Scelionidae), was established in Hawai‘i to control the southern green stink bug *Nezara viridula* Linnaeus (Hemiptera: Pentatomidae) (Davis and Chong 1969). The

establishment of these parasitoids on O‘ahu yielded good results in the control of *N. viridula* with *T. basalis* producing high percentages of parasitism under many circumstances (Davis 1964).

Yoshimoto (1965) completed a synopsis of Eulophidae in Hawai‘i, containing keys to 33 genera and 71 species, and descriptions of four new species and a genus new to science (See Appendix for egg parasitoid species). A synopsis for the Encyrtidae of Hawai‘i described both native and introduced species (Beardsley 1976). Five species of egg parasitoids were listed in these synopses, falling into two genera: *Ooencyrtus* (Encyrtidae) and *Ootetrastichus* (Eulophidae), all non-native (Appendix).

In 1976 the banana skipper (Lepidoptera: *Erionota thrax*) was detected in Hawai‘i and a new egg parasitoid: *Ooencyrtus pallidipes* (Ashmead) (Hymenoptera: Encyrtidae), was introduced to aid in controlling this species. This egg parasitoid was successfully established and field data revealed it to be an effective biocontrol agent on this new invasive pest (Mau et al. 1980).

In 1981 an unidentified species of *Palaeomymar*, an egg parasitoid belonging to the then newly described family Mymarommatidae was collected on Moloka‘i, being the first of this new family to be collected in the North Pacific (Beardsley et al. 2000). A set of three keys was published in 2000 to organize Mymaridae; the first key separated 17 genera (Beardsley and Huber 2000), the second key listed 11 species in the genus *Gonatocerus* (Huber and Beardsley 2000), and the third key listed 12 species of *Anagrus* (Triapitsyn and Beardsley 2000).

Another egg parasitoid survey was conducted in the Hawai‘i Volcanoes National Park from 1997 – 1998 to investigate parasitism on *Sophonia rufofascia,* Kuoh & Kuoh (Hemiptera:

Cicadellidae). A total of 1,055 individual hymenopteran parasitoids from 5 families: Mymaridae, Encyrtidae, Trichogrammatidae, Eulophidae, and one unknown were collected during this study. Unfortunately none of these parasitoids were identified to species, but over 95% of the egg parasitoids found came from Mymaridae (Johnson et al. 2001).

Johnson (2004) also investigated parasitism and mortality of the endemic koa bug *Coleotichus blackburniae* White (Hemiptera: Scuttelleridae) by collecting parasitized eggs from the field. Two introduced species of egg parasitoids were identified: *Telenomus (Trissolcus) basalis*, and *Anastus* sp. (Eupelmidae), but the impact of these introduced parasitoids was low with only a few sites yielding a maximum parasitism rate of 40% on non-native plants (Johnson et al. 2005).

In 2011 a survey for natural enemies of *Helicoverpa zea* was done on O‘ahu and found a new species of Trichogramma: *T. achaeae.* This new species was also collected on the island of Kaua‘i from eggs of Lycaenidae and eggs of *Achaea janata* (L.) (Lepidoptera: Noctuidae) (Wright and Stouthamer 2011).

The largest family of egg parasitoids in Hawai‘i is Mymaridae with 73 species. Most of the species in this family (56) are non-native (47 adventives, 9 purposefully introduced) but this family also includes the greatest number of endemic species of egg parasitoids in the islands (17) with one species having an unknown origin. Almost all of the endemic species are in the genus *Polynema* with only two belonging to the genus *Anagrus*. One of the native *Anagrus* species targets eggs from Odonata, with one of the host genera an endemic damselfly: *Megalagrion* (Triapitsyn and Beardsley 2000). No species-specific host records were found for the endemic species of *Polynema*, but all of the endemic mymarids except for *A. insularis* and *A. oahuensis*

are known to attack eggs from Hemiptera. The remaining seven species of Mymaridae are thought to be adventive, but the true origin is not known (Appendix).

Micronesia:

Micronesia is a subregion of Oceania that includes the Marianas, Guam, Carolines, Marshalls, Gilberts, Ocean, Narau, Wake, Marcus, Bonins, and the Volcano Islands. Even though the subregion houses many island chains, the total land area is only 702 km2. More than 100 species of non-native organisms have been introduced into this archipelago since 1911 for the control of pests, especially on the main island of Guam. However a majority of these introductions failed to establish and of the parasitoids that did get established, only a few are egg parasitoids (Table 1.6) (Nafus 1993). In total there are 27 species of egg parasitoids known from Micronesia. Of these species only nine are native which are split between two families: Mymaridae (5 natives) and Trichogrammatidae (4 natives). The majority of the egg parasitoids are adventive (18), while six were purposefully introduced, and four of unknown origin.

In 1935 *Trichogramma chilonis*, Ishii, was introduced to Guam for the control of the sugarcane stem borer: *Eucosoma schistoceana* Snellan (Lepidoptera: Pyralidae) and was found parasitizing eggs of *Daphnis nerii*, L. (Lepidoptera: Sphingidae) along with two other species of egg parasitoids: *Eupelmis* sp, and *Ooencyrtus* nr. *papilionis* (Table 1.5) (Moore and Miller 2008).

In 1946 a catalogue of insect collections from Guam by Fullaway, Swezey, and Usinger documented eight egg parasitoids: *Ooencyrtus pacificus* Waterston (Hymenoptera: Encyrtidae),

Anastus picticornis (Cameron) (Hymenoptera: Eupelmidae), *Alaptus caecilii* Girault (Hymenoptera: Mymaridae), *Anagrus flaveolus* Waterhouse (Hymenoptera: Mymaridae), *Anagrus optabilis* Perkins (Mymaridae), *Mymarilla tyndalli* (Girault) (Hymenoptera: Mymaridae), *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), *T. nanum* Zehntner (Table 1.5) (Bernice 1946).

In 1954 a survey was conducted in part by the Bishop Museum, United States Office of Naval Research, and The National Science Foundation to catalogue the insect fauna in Micronesia from the Marianas, Carolines, Marshalls, Gilberts, Ocean, Narau, Wake, Marcus, Bonins, and the Volcano Islands (Gressitt 1954). From this survey 29 egg parasitoid species were collected (Table 1.5). The families with the greatest number of species were Mymaridae and Trichogrammatidae (Doutt 1955). Egg parasitoids in the families Eulophidae, Encyrtidae, and Evaniidae were also found in this survey (Table 1.5) (Townes 1958; Yoshimoto 1965).

A study in 1993 surveyed egg masses of two butterfly species on Guam: *Hypolimas bolina* L. (Lepidoptera: Nymphalidae) and *H. anomala* L., to investigate the non-target impacts of introduced predators and parasitoids (Nafus 1993). That study found three different egg parasitoids on both species of butterflies, two of which were suspected of being native: *Telenomus sp*. (Hymenoptera: Scelionidae) and *Ooencyrtus sp*. (Hymenoptera: Encyrtidae). The following introduced egg parasitoids were also found: *T. chilonis*, *T. tortricis*, Girault, and *Brachymeria lasus,* Walker (Hymenoptera: Chalcidae). The apparently native *Telenomus* species was responsible for the highest parasitism rate in both *H. bolina* (66%) and *H. anomala* (47%), with the potentially native *Ooencyrtus* producing 21% and 17% parasitism respectively. The introduced *T. chilonis* caused 12% and 36% parasitism respectively. It was concluded that non-target impacts from the introduced parasitoids do not pose a threat to the butterfly population on

Guam.

The largest family of egg parasitoids within Micronesia is Trichogrammatidae (13 species) with Mymaridae coming in close second at 11 species. Both of these families have most of their species documented as adventive with Hemipteran hosts. It would be interesting to see an egg parasitoid survey on native hemipterans to determine the effects if any from these adventive parasitoids. Currently there is no indication that any of these hemipteran parasitoids are causing harm to the native fauna of Micronesian islands, but there are also no documented studies with the purpose of looking at native hemipteran eggs.

Conclusions:

The purpose of this literature review was to explore and summarize the history and uses of egg parasitoids across island archipelagos. Although egg parasitoids have been employed for agricultural practices in many island groups, there remains a lack of information on the impacts of parasitoid introductions to these island systems. Comparatively more studies and surveys have been undertaken to understand the impacts of parasitoids in Hawai‘i, partly due to its high level of endemism and high numbers of introductions (e.g., King et al. 2010; Kier et al. 2009; Messing and Wright 2006; Johnson et al. 2005; Peck et al. 1998; Funasaki et al. 1988). The majority of research undertaken on invasive species in island systems has been focused on organisms other than insects (Simberloff 1995).

The families with the greatest number of species documented throughout the archipelagos studied here were Mymaridae, Scelionidae, Evaniidae and Trichogrammatidae. However, there remain many archipelagos yet to be surveyed, such as the Kerguelen and Mascarene islands of

the Indian Ocean. Within the surveyed archipelagos, additional species continue to be detected, as evidenced by the many updates to previous faunal catalogues of the various island chains. It would be beneficial to focus on detecting and assessing the impacts of introduced egg parasitoids on non-target native insect communities in island ecosystems because often no follow-up studies are undertaken on introduced species, beyond those on their impacts on the targeted pests (Howarth 1991; King et al. 2010; Boettner et al. 2000).

The diversity of egg parasitoids in the different archipelagos is summarized in Table 1.7. Out of the introduced egg parasitoids in this study, Hawai‘i had the greatest number of successful establishments (26) and the largest number that contributed to post-release suppression of pests (Table 1.5). Micronesia had the second largest number of introduced egg parasitoids (6). The family of egg parasitoids producing the greatest number of successful biological control agents throughout the island systems in this study is Mymaridae, which appears to have success across a broad range of host orders: Hemiptera, Psocoptera, Diptera and Coleoptera. Mymaridae was also the most abundant family of egg parasitoid across the archipelagos in this review with the exclusion of the Micronesia, which had three more trichogrammatids than mymarids. However, there were more native mymarids within the Micronesia than native trichogrammatids which isn't surprising due to the common practice of using *Trichogramma* in bio control (Smith 1996). The Galapagos had no native egg parasitoids recovered which is really interesting because it is known for being a well-protected nature reserve, but it also had no purposeful introductions of egg parasitoids.

The island system with the greatest number of native species is Hawai‘i with a total of 33 species. Micronesia had the second greatest number of native species with a total of nine. An interesting point to note is that the Micronesia has the smallest total area when compared to the

other archipelagos in this review, yet it had the second largest number of native species. Micronesia also had the second greatest number of egg parasitoids overall, with Hawai‘i having the most. There doesn’t appear to be any correlation between island land mass and egg parasitoid diversity, but nothing definitive can be stated about this. Micronesia holds very remote island chains, which could explain the greater number of native species owing to the fact it doesn’t have any close neighboring landmasses for species to continually migrate from.

A few interesting observations are that the Azores had no egg parasitoids documented from the family Evaniidae while all the other archipelagos did, and there are no Trichogrammatidae recorded from French Polynesia. The Azores and Canary Islands had no documented purposeful introductions of egg parasitoids while the other three archipelagos had multiple. It should be noted that even though there has been no documentation of certain groups of egg parasitoids within some archipelagos, that does not necessarily mean those parasitoids are not there. It is very possible that a lack of documentation is due to a lack of effort put into searching. The Canary Islands had many more adventive species than the Azores which makes sense because it is located much closer to the continents of Africa and Europe than the Azores. Another interesting observation is that even though the Azores and Canary Islands are neighboring Archipelagos, they only shared two species of egg parasitoids: *Baes seminulum* (Scelionidae) and *Mymar taprobanicum* (Mymaridae) and *M. taprobanicum* is listed as native to the Azores and adventive to the Canary Islands, which indicates that *M. taprobanicum* reached the Canary Islands from the Azores by an accidental introduction.

It is of great conservation importance to search for potential negative impacts of introduced parasitoids on native fauna and flora, and this is especially true in island ecosystems due to their unique ecosystems and high levels of endemism (Mueller-Dombois and Loope 1990; Kier et al.

2009). This is notably true in island ecosystems that have already suffered considerable habitat degradation and development that has resulted in significant reduction in the ranges and populations of native insects, whose continued survival is particularly sensitive to the threat of parasitization by non-specific exotic biocontrol agents.

Table 1.1 Egg parasitoids of the Azores

Family	Genus	Species	Author	Reference	Origin	Order of Host
Mymaridae	*Anaphes*	*sp.*		Pintureau et al. (1990)	Unknown	Coleoptera
Mymaridae	*Litus*	*cynipseus*	Haliday	BIOTA Azores (2019)	Native	Coleoptera
Mymaridae	*Mymar*	*taprobanicum*	Haliday	BIOTA Azores (2019)	Native	Hemiptera
Mymaridae	*Ooctonus*	*sp.*		Pintureau et al. (1990)	Unknown	Diptera
Scelionidae	*Baeus*	*seminulum*	Haliday	BIOTA Azores (2019)	Native	Chelicerata
Scelionidae	*Gryon*	*missellum.*	Haliday	Pintureau et al. (1990)	Unknown	Lepidoptera
Scelionidae	*Idris*	*rufescens*	(Kieffer)	BIOTA Azores (2019)	Native	Chelicerata
Scelionidae	*Telenomus*	*angustatus*	(Thomson)	Pintureau et al. (1990)	Unknown	Lepidoptera
Trichogrammatidae	*Trichogramma*	*achaeae*	Nagaraja &Nagarkatti	Cabello et al. (2009)	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*cordubensis*	Vargas & Cabello	Pintureau et al. (1990)	Adventive	Lepidoptera

Table 1.2 Egg parasitoids of the Canary Islands

Family	Genus	Species	Author	Reference	Origin	Order of Host
Eupelmidae	*Anastus*	*giraudi*	Ruschka	Báez et al. (2004)	Unknown	Orthoptera
Eupelmidae	*Anastus*	*magnoculus*	Askew	Askew & Nieves (2004)	Native	Unknown
Evaniidae	*Evania*	*appendigaster*	Linnaeus	Báez et al. (2004)	Native?	Blattodea
Evaniidae	*Prosevania*	*fuscipes*	Illiger	Báez et al. (2004)	Native?	Blattodea
Mymaridae	*Anagrus*	*atomus*	Linnaaeus	Kopenen et al. (2016)	Adventive	Hemiptera
Mymaridae	*Cleruchus*	*pluteus*	Enock	Kopenen et al. (2016)	Adventive	Coleoptera

Table 1.3 Egg parasitoids of French Polynesia

Family	Genus	Species	Author	Reference	Origin	Order of Host
Evaniidae	*Evania*	*appendigaster*	Linnaeus	Cheeseman (1928)	Pur. Introduced	Blattodea
Evaniidae	*Evania*	*sericea*	Cameron	Cheeseman (1928)	Adventive	Blattodea
Mymaridae	*Anagrus*	*baeri*	Girault	Ramage (2017)	Adventive	Hemiptera
Mymaridae	*Anagrus*	*frequens*	Perkins	Ramage (2017)	Adventive	Hemiptera
Mymaridae	*Anagrus*	*iti*	Triapitsyn	Ramage (2017)	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*ashmeadi*	Girault	Petit et al. (2008)	Pur. Introduced	Hemiptera
Mymaridae	*Gonatocerus*	*dolichocerus*	Ashmead	Ramage (2017)	Pur. Introduced	Hemiptera
Mymaridae	*Stephanodes*	*reduvioli*	Perkins	Ramage (2017)	Adventive	Hemiptera
Scelionidae	*Telenomus*	*mataieaensis*	Fouts	Ramage (2017)	Endemic	Unknown
Scelionidae	*Telenomus*	*mumfordi*	Fouts	Ramage (2017)	Endemic	Unknown
Scelionidae	*Trissolcus*	*personatus*	Johnson	Ramage (2017)	Adventive	Hemiptera

Table 1.4 Egg parasitoids of the Galapagos

Family	Genus	Species	Author	Reference	Origin	Order of Host
Aphilinidae	*Centrodora*	*mireyae*	(DeSantis)	Peck et al. 1998	Adventive	Unknown
Aphilinidae	*Centrodora*	*perkinsi*	(Waterston)	Peck et al. 1998	Adventive	Unknown
Evaniidae	*Evania*	*appendigaster*	Lineaeus	Peck et al. 1998	Adventive	Blattodea
Scelionidae	*Macrotelia*	*absona*	Muesebeck	Peck et al. 1998	Adventive	Orthoptera
Scelionidae	*Trissolcus*	*teretis*	Johnson	Peck et al. 1998	Adventive	Lepidoptera

Table 1.5 (1 of 5). Egg parasitoids of Hawai‘i

Family	Genus	Species	Author	Origin	Host Order
Aphelinidae	*Centrodora*	*xiphidii*	(Perkins)	Adventive	Orthoptera
Braconidae	*Biosteres*	*arisanus*	Sonan	Pur. Introduced	Diptera
Braconidae	*Chelonus*	*blackburni*	Cameron	Adventive	Lepidoptera
Encyrtidae	*Ooencyrtus*	*erionotae*	Ferriere	Pur. Introduced	Lepidoptera
Encyrtidae	*Ooencyrtus*	*guamensis*	Fullaway	Adventive	Lepidoptera
Encyrtidae	*Ooencyrtus*	*johnsoni*	(Howard)	Pur. Introduced	Hemiptera
Eulophidae	*Ootetrastichus*	*beatus*	Perkins	Pur. Introduced	Hemiptera
Eulophidae	*Ootetrastichus*	*formosanus*	Timberlake	Pur. Introduced	Hemiptera
Eupelmidae	*Anastatus*	*koebelei*	Ashmead	Endemic	Orthoptera
Eupelmidae	*Anastatus*	*picticornis*	(Cameron)	Adventive	Orthoptera
Eupelmidae	*Anastatus*	*tenuipes*	Bolivar & Pieltain	Adventive	Orthoptera
Eupelmidae	*Eupelmus*	*axestops*	Perkins	Endemic	Orthoptera
Eupelmidae	*Eupelmus*	*rhynocogoni*	Perkins	Native	Coleoptera
Evaniidae	*Evania*	*appendigaster*	(Linnaeus)	Adventive	Blattodea
Evaniidae	*Szepligetella*	*sericea*	(Cameron)	Adventive?	Blattodea
Mymaridae	*Acmopolynema*	*uma*	Schauff	Adventive	Unknown
Mymaridae	*Alaptus*	*globosicornis*	Girault	Adventive	Psocoptera
Mymaridae	*Alaptus*	*immaturus*	Perkins	Adventive	Psocoptera
Mymaridae	*Alaptus*	*sp. 1*	Beardsley & Huber	Adventive?	Psocoptera
Mymaridae	*Alaptus*	*sp. 2*	Beardsley & Huber	Adventive?	Psocoptera
Mymaridae	*Alaptus*	*sp. 3*	Beardsley & Huber	Adventive?	Psocoptera
Mymaridae	*Alaptus*	*sp. 4*	Beardsley & Huber	Adventive?	Psocoptera
Mymaridae	*Alaptus*	*sp. 5*	Beardsley & Huber	Adventive?	Psocoptera
Mymaridae	*Alaptus*	*sp. 6*	Beardsley & Huber	Adventive	Psocoptera
Mymaridae	*Alaptus*	*sp. 7*	Beardsley & Huber	Adventive	Psocoptera
Mymaridae	*Anagrus*	*armatus*	Ashmead	Pur. Introduced	Hemiptera
Mymaridae	*Anagrus*	*?columbi*	Perkins	Adventive	Odonata
Mymaridae	*Anagrus*	*empoascae*	Dozier	Adventive	Hemiptera

Table 1.5 (continued, 2 of 5). Egg parasitoids of Hawai‘i

Mymaridae	*Anagrus*	*frequens*	Perkins	Pur. Introduced	Hemiptera
Mymaridae	*Anagrus*	*insularis*	Dozier	Endemic	Odonata
Mymaridae	*Anagrus*	*nigriventis*	Girault	Adventive	Hemiptera
Mymaridae	*Anagrus*	*oahuensis*	Triapitsyn & Beardsley	Endemic	Unknown
Mymaridae	*Anagrus*	*optabilis*	Perkins	Pur. Introduced	Hemiptera
Mymaridae	*Anagrus*	*perforator*	(Perkins)	Pur. Introduced	Hemiptera
Mymaridae	*Anagrus*	*sp. 1*	Triapitsyn & Beardsley	Adventive?	Hemiptera
Mymaridae	*Anagrus*	*sp. 2*	Triapitsyn & Beardsley	Adventive?	Hemiptera
Mymaridae	*Anagrus*	*takeyanus*	Gordh	Adventive	Hemiptera
Mymaridae	*Anagrus*	*yawi*	Fullaway	Pur. Introduced	Hemiptera
Mymaridae	*Anaphes*	*calendrae*	(Gahan)	Pur. Introduced	Coleoptera
Mymaridae	*Arescon*	*peregrinus*	(Perkins)	Adventive	Unknown
Mymaridae	*Camptoptera*	*sp.1*	Beardsley & Huber	Adventive	Coleoptera?
Mymaridae	*Camptoptera*	*sp. 2*	Beardsley & Huber	Adventive	Coleoptera?
Mymaridae	*Camptoptera*	*sp. 3*	Beardsley & Huber	Adventive	Coleoptera?
Mymaridae	*Chaetomymar*	*bagicha*	(Narayanan, Subba Rao & Kaur)	Adventive	Hemiptera
Mymaridae	*Cleruchus*	*sp.*	Beardsley & Huber	Adventive	Unknown
Mymaridae	*Dicopus*	*psyche*	Girault	Adventive	Unknown
Mymaridae	*Erythmelus*	*funiculi*	(Annecke & Doutt)	Adventive	Hemiptera
Mymaridae	*Erythmelus*	*sp. 1*	Beardsley & Huber	Adventive	Hemiptera
Mymaridae	*Erythmelus*	*sp. 2*	Beardsley & Huber	Adventive	Hemiptera
Mymaridae	*Erythmelus*	*sp. 3*	Beardsley & Huber	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*californicus*	Girault	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*capitatus*	Gahan	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*dolichocerus*	Ashmead	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*ornatus*	Gahan	Adventive?	Hemiptera
Mymaridae	*Gonatocerus*	*pygmaeus*	Girault	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*sp. 1*	Huber & Beardsley	Adventive	Hemiptera

Table 1.5 (continued, 3 of 5). Egg parasitoids of Hawai‘i

Mymaridae	Gonatocerus	sp. 2	Huber & Beardsley	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*sp. 3*	Huber & Beardsley	Adventive	Hemiptera
Mymaridae	*Gonatocerus*	*sp. 4*	Huber & Beardsley	Adventive	Hemiptera
Mymaridae	*Gonatocerus.*	*membraciphagus*	Beardsley	Adventive	Hemiptera
Mymaridae	*Gonatocerus.*	*mexicanus*	Perkins	Adventive	Hemiptera
Mymaridae	*Kikiki*	*huna*	Huber & Beardsley	Adventive	Unknown
Mymaridae	*Mymar*	*taprobanicum*	Ward	Adventive	Hemiptera
Mymaridae	*Ooctonus*	*sp.*	Beardsley & Huber	Adventive	Unknown
Mymaridae	*Palaeomymar*	*sp.*	Beardsley & Huber	Adventive	Unknown
Mymaridae	*Paranagrus*	*osborni*	Fullaway	Pur. Introduced	Hemiptera
Mymaridae	*Polynema*	*apicalis Perkins, 1910*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*ciliatum Perkins, 1910*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*ema*	Schauff & Grissell	Pur. Introduced	Hemiptera
Mymaridae	*Polynema*	*gigas*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*hawaiiensis Ashmead, 1901*	end	Endemic	Hemiptera
Mymaridae	*Polynema*	*jassidarum*	end	Endemic	Hemiptera
Mymaridae	*Polynema*	*nanum*	end?	Endemic?	Hemiptera
Mymaridae	*Polynema*	*oahuensis*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*perforator*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*poeta*	Girault	Endemic	Hemiptera
Mymaridae	*Polynema*	*pyrophila*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*rubriventre*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*saga*	(Girault)	Adventive	Hemiptera
Mymaridae	*Polynema*	*scrutator*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*sp.*	Perkins	Pur. Introduced	Hemiptera
Mymaridae	*Polynema*	*tantalea longipenne*	Girault	Endemic	Hemiptera
Mymaridae	*Polynema*	*tantalea tantalea*	Perkins	Endemic	Hemiptera
Mymaridae	*Polynema*	*terrestris*	Perkins	Endemic	Hemiptera

Table 1.5 (continued, 4 of 5). Egg parasitoids of Hawai‘i continued

Mymaridae	*Polynema*	*triscia*	Perkins	Endemic	Hemiptera
Mymaridae	*Schizophragma*	*bicolor*	Dozier	Adventive	Hemiptera
Mymaridae	*Stephanodes*	*reduvioli*	(Perkins)	Adventive	Hemiptera
Mymaridae	*Stethynium*	*sp. 1*	Beardsley & Huber	Adventive	Hemiptera
Mymaridae	*Stethynium*	*triclavatum*	Enock	Adventive	Hemiptera
Mymarommatidae	*Palaeomymar*	*sp.*	Meunier	Unknown	Unknown
Platygastridae	**Aphanomerus*	*pusillus*	Perkins	Pur. Introduced	Hemiptera
Pteromalidae	*Scutellista*	*cyanea*	Motsch	Pur. Introduced	Coccidae
Scelionidae	*Anteromorpha*	*dubiosa*	Perkins	Adventive	Unknown
Scelionidae	*Aphanomerus*	*pusillus*	Perkins	Pur. Introduced	Hemiptera
Scelionidae	*Baeus*	*latrodecti*	Dozier	Pur. Introduced	Hemiptera
Scelionidae	*Baeus*	*persordidus*	Perkins	Adventive?	Hemiptera
Scelionidae	*Baryconus*	*sp.*	Beardsley	Adventive	Unknown
Scelionidae	*Caenoteleia*	*elegans*	Perkins	Adventive	Orthoptera
Scelionidae	*Calliscelio*	*exul*	Perkins	Adventive	Orthoptera
Scelionidae	*Dyscritobaeus*	*comitans*	Perkins	Adventive?	Hemiptera
Scelionidae	*Encyrtoscelio*	*sp.*	Beardsley	Adventive	Unknown
Scelionidae	*Idris*	*peregrinus*	Perkins	Endemic	Spiders
Scelionidae	*Opisthacantha*	*hawaiiensis*	Ashmead	Endemic?	Scorpions
Scelionidae	*Opisthacantha*	*montana*	Perkins	Endemic?	Scorpions
Scelionidae	*Opisthacantha*	*nigricornis*	Ashmead	Endemic?	Scorpions
Scelionidae	*Opisthacantha*	*oahuensis*	Perkins	Endemic?	Scorpions
Scelionidae	*Opisthacantha*	*perkinsi*	Ashmead	Endemic?	Scorpions
Scelionidae	*Opisthacantha*	*tarsalis*	Ashmead	Endemic?	Scorpions
Scelionidae	*Scelio*	*pembertoni*	Timberlake	Pur. Introduced	Orthoptera
Scelionidae	*Telenomus*	*adelphus*	Perkins	Adventive?	Hemiptera
Scelionidae	*Telenomus*	*despiciendus*	Perkins	Adventive?	Hemiptera
Scelionidae	*Telenomus*	*dignus*	(Gahan)	Pur. Introduced	Lepidoptera
Scelionidae	*Telenomus*	*nawai*	Ashmead	Adventive	Hemiptera
Scelionidae	*Telenomus*	*paractias*	Perkins	Adventive?	Hemiptera
Scelionidae	*Telenomus*	*rhopali*	Perkins	Adventive?	Hemiptera

Table 1.5 (continued, 5 of 5). Egg parasitoids of Hawai‘i

Scelionidae	*Telenomus*	*sp.*	Beardsley	Adventive	Unknown
Scelionidae	*Telenomus*	*vulcanus*	Perkins	Adventive	Thysanoptera
Scelionidae	*Trissolcus*	*basalis*	(Wollaston)	Pur. Introduced	Hemiptera
Scelionidae	*Trissolcus*	*brochymenae*	(Ashmead)	Pur. Introduced	Hemiptera
Scelionidae	*Trissolcus*	*crypticus*	Clarke	Pur. Introduced	Hemiptera
Scelionidae	*Trissolcus*	*mitsukurii*	(Ashmead)	Pur. Introduced	Hemiptera
Trichogrammatidae	*Aphelinoidea*	*xenos*	Timberlake	Adventive?	Hemiptera
Trichogrammatidae	*Brachistella*	*lutea*	(Fullaway)	Native	Hemiptera
Trichogrammatidae	*Megaphragma*	*mymaripenne*	Timberlake	Adventive	Thysanoptera
Trichogrammatidae	*Oligosita*	*caerulocephala*	(Fullaway)	Adventive?	Hemiptera
Trichogrammatidae	*Oligosita*	*elimaeae*	Viggiani	Adventive	Hemiptera
Trichogrammatidae	*Oligosita*	*hawaiiana*	Viggiani	Adventive?	Hemiptera
Trichogrammatidae	*Oligosita*	*hilaris*	(Perkins)	Adventive?	Hemiptera
Trichogrammatidae	*Paracentrobia*	*lutea*	(Fullaway)	Adventive	Hemiptera
Trichogrammatidae	*Paracentrobia*	*perditrix*	(Gahan)	Adventive?	Hemiptera
Trichogrammatidae	*Trichogramma*	*achaeae*	Nagaraja & Nagarkatti	Adventive	Coleoptera
Trichogrammatidae	*Trichogramma*	*beardsleyi*	nomen nudum?	Adventive?	Lepidoptera
Trichogrammatidae	*Trichogramma*	*chilonis*	Iishii	Pur. Introduced	Lepidoptera
Trichogrammatidae	*Trichogramma*	*evanescens*	Westwood	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*higai*	Oatman & Platner	Adventive?	Lepidoptera
Trichogrammatidae	*Trichogramma*	*japonicum*	Ashmead	Pur. Introduced	Lepidoptera
Trichogrammatidae	*Trichogramma*	*minutum*	Riley	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*ostriniae*	Pang & Cheng	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*papilionis*	Nagarkatti	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*perkinsi*	Girault	Endemic	Lepidoptera
Trichogrammatidae	*Trichogramma*	*pretiosum*	Riley	Adventive?	Lepidoptera
Trichogrammatidae	*Trichogramma*	*sembeli*	Oatman & Platner	Endemic?	Lepidoptera
Trichogrammatidae	*Trichogramma*	*semifumatum*	Pinto et al.,	Endemic	Lepidoptera
Trichogrammatidae	*Trichogramma*	*vargasi*	Oatman & Platner	Endemic	Lepidoptera
Trichogrammatidae	*Ufens*	*elimaeae*	Timberlake	Adventive?	Hemiptera
Trichogrammatidae	*Uscana*	*semifumipennis*	Girault	Adventive	Coleoptera

Table 1.6 (1 of 3). Egg parasitoids of Micronesia

Family	Genus	Species	Author	Reference	Origin	Order of Host
Chalcidae	*Brachymeria*	*lasus*	Walker	Nafus (1993)	Pur. Introduced	Lepidoptera
Encyrtidae	*Ooencyrtus*	*pacificus*	Waterston	Bernice (1946)	Adventive	Hemiptera
Encyrtidae	*Ooencyrtus*	*nr. papilionis*	Ashmead	Moore & Miller (2008)	Adventive	Lepidoptera
Encyrtidae	*Ooencyrtus*	*swezeyi*	Bernice	Yoshimoto (1965)	Adventive	Lepidoptera
Encyrtidae	*Ooencyrtus*	*sp.*		Nafus (1993)	Native?	Lepidoptera
Eulophidae	*Ootetrastichus*	*beatus*	Perkins	Yoshimoto (1965)	Adventive	Hemiptera
Eulophidae	*Ootetrastichus*	*formosanus*	Timberlake	Yoshimoto (1965)	Adventive	Hemiptera
Eupelmidae	*Anastus*	*picticornis*	(Cameron)	Bernice (1946)	Unknown	Blattodea
Eupelmidae	*Eupelmus*	*sp.*		Moore & Miller (2008)	Unknown	Lepidoptera
Evaniidae	*Evania*	*appendigaster*	(Linnaeus)	Townes (1958)	Pur. Introduced	Blattodea
Evaniidae	*Prosevania*	*variiceps*	(Kieffer)	Townes (1958)	Pur. Introduced	Blattodea
Evaniidae	*Szepligetella*	*sericea*	(Cameron)	Townes (1958)	Pur. Introduced	Blattodea
Mymaridae	*Alaptus*	*caecilii*	Girault	Bernice (1946) Doutt (1955)	Adventive	Psocoptera

Table 1.6 (continued, 2 of 3). Egg parasitoids of Micronesia

Mymaridae	*Anagrus*	*flaveolus*	Waterhouse	Bernice (1946) Doutt (1955)	Adventive	Hemiptera
Mymaridae	*Anagrus*	*optabilis*	Perkins	Bernice (1946) Doutt (1955)	Adventive	Hemiptera
Mymaridae	*Arescon*	*clarkei*	Doutt	Doutt (1955)	Adventive	Hemiptera
Mymaridae	*Lymaenon*	*saipanensis*	Doutt	Doutt (1955)	Native	Hemiptera
Mymaridae	*Lymaenon*	*sp.*		Doutt (1955)	Native	Hemiptera
Mymaridae	*Lymaenon*	*sp.*		Doutt (1955)	Native	Hemiptera
Mymaridae	*Mymarilla*	*tyndalli*	(Girault)	Bernice (1946) Doutt (1955)	Adventive	Hemiptera
Mymaridae	*Nesetaerus*	*gressitti*	Doutt	Doutt (1955)	Native	Unknown
Mymaridae	*Polynema*	*sp.*		Doutt (1955)	Native	Hemiptera
Mymaridae	*Stephanodes*	*similis*	Foerster	Doutt (1955)	Adventive	Hemiptera
Scelionidae	*Telenomus*	*sp.*		Nafus (1993)	Native?	Lepidoptera
Trichogrammatidae	*Abella*	*mira*	Girault	Doutt (1955)	Adventive	
Trichogrammatidae	*Aphelinoidea*	*mariana*	Doutt	Doutt (1955)	Native	Hemiptera
Trichogrammatidae	*Aphelinoidea*	*oceanica*	Timberlake	Doutt (1955)	Adventive	Hemiptera

Table 1.6 (continued, 3 of 3). Egg parasitoids of Micronesia

Trichogrammatidae	*Haeckeliania*	*brontispae*	Ferrière	Doutt (1955)	Adventive	Coleoptera
Trichogrammatidae	*Lathromeris*	*pacifica*	Doutt	Doutt (1955)	Native	Coleoptera
Trichogrammatidae	*Oligosita*	*hilaris*	(Perkins)	Doutt (1955)	Adventive	Hemiptera
Trichogrammatidae	*Oligosita*	*kusaiensis*	Doutt	Doutt (1955)	Native	Hemiptera
Trichogrammatidae	*Oligosita*	*oceanica*	Doutt	Doutt (1955)	Native	Hemiptera
Trichogrammatidae	*Oligosita*	*utilis*	Kowalski	Doutt (1955)	Adventive	Hemiptera
Trichogrammatidae	*Trichogramma*	*chilonis*	Ishii	Doutt (1955) Nafus et al. (1989) Nafus (1993) Moore & Miller (2008)	Pur. Introduced	Lepidoptera
Trichogrammatidae	*Trichogramma*	*minutum*	Riley	Bernice (1946) Doutt (1955)	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*nanum*	(Zehntner)	Bernice (1946) Doutt (1955)	Adventive	Lepidoptera
Trichogrammatidae	*Trichogramma*	*tortricis*	Girault	Doutt (1955) Nafus (1993)	Pur. Introduced	Lepidoptera

Table 1.7 Summary of egg parasitoid diversity and origins in the reviewed archipelagos

		Number of egg parasitoid species				
Archipelago	Total land area (km2)	Native	Adventive	Purposefully Introduced	Unknown Status	Total
Azores	2347	4	2	0	4	10
Canary Islands	7493	3	12	0	8	23
French Polynesia	4167	2	6	3	0	11
Galapagos	7880	0	5	0	0	5
Hawaiian Islands	16636	33	63	25	26	146
Micronesia	702	9	18	6	4	27

Chapter 2. Variation in Lepidoptera Egg Parasitism across an elevational gradient on O‘ahu, Hawai‘i

Abstract

Within the past century alone over 700 species of parasitoids have been introduced to the Hawaiian Islands. Egg parasitoids, in particular, are used regularly in lepidopteran pest control projects, but little is known about the egg parasitoid fauna of Hawai‘i because their small size makes them hard to detect. I conducted a multi-year, multi-elevational survey to find established and potentially newly introduced lepidopteran egg parasitoids using sentinel eggs of three moths: *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Daphnis nerii* Linnaeus (Lepidoptera: Sphingidae) and *Agrius cingulata* Fabricius (Lepidoptera: Sphingidae). In this study 3,350 sentinel eggs were deployed over elevational gradients on the two mountain ranges of O‘ahu with only 63 eggs parasitized (3.1%) and 35 eggs predated (1.7%). Three parasitoids were reared from sentinel eggs: *Ooencyrtus pallidipes* (Ashmead) (Hymenoptera: Encyrtidae), *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and an unidentified wasp, but only *T. chilonis* was found in native forests at high elevations. A significant difference was found in the variety of alien parasitoid species attacking *D. nerii* and *H. zea,* with *D. nerii* being attacked by all three.

Introduction

The Hawaiian archipelago has borne the brunt of globalization and is known as the invasive species capital of the world, with more introduced species than anywhere else on the planet (Beardsley, 1962, 1979; Messing & Wright, 2006). Many nonnative species entered

Hawai‘i in the late 1800’s as a result of the islands being on major trade routes and a whaling center. New crops for commercial agriculture were being introduced and many exotic plant species along with their insect hitchhikers established as a result (Funasaki et al. 1988). Among these introduced insects were parasitoids, probably already infesting their hosts, who themselves were accidentally imported. Many other parasitoids are purposefully introduced for protection of crops from pest insects; over 700 species of parasitoids and predators have been introduced to Hawai‘i to control invertebrate pests in the past century alone (Johnson et al. 2005). The exact number of invasive parasitoids currently established from introductions in Hawai‘i is not known, but this is not surprising because many programs for biological control are not monitored or well documented, (Boettner et al. 2000; Howarth, 1991; King et al. 2010) and there have been few surveys targeting accidentally introduced parasitoid occurrence in the islands.

Parasitoids are organisms that use other organisms as hosts for their larvae, killing the host in the process. Most insect parasitoids belong to the orders Hymenoptera and Diptera, and many tend to be relatively specific in their choice of host, making them very useful for biological control. Egg parasitoids are a lesser known group of parasitoid biocontrol as compared to larval parasitoids such as Ichneumonidae and Braconidae, but can be important in biological control, especially the superfamilies Platygastroidea and Chalcidoidea. Because Hawai‘i has been the subject of so many intentional and accidental introductions, the fauna of important but inconspicuous taxa like egg parasitoids, is poorly understood. Yet, these minute wasps can have significant impacts on both pest species and non-target native insects (Babendreier et al. 2003b, 2003a; Smith, 1996) and documenting the egg parasitoids established in Hawai‘i is of broad interest.

In Hawai‘i there are at least 147 known egg parasitoids (see Chapter 1) mostly belonging to three families: Mymaridae, Scelionidae, and Trichogrammatidae. The largest family is Mymaridae with 74 documented species including 18 species endemic to Hawai‘i (Chapter 1). However, none of the mymarids target Lepidoptera. Out of all the egg parasitoids in Hawai‘i only 17 are known to target Lepidoptera, and most of these (13) are trichogrammatids. The other four known egg parasitoids for Lepidoptera are two encyrtids, one braconid, and a scelionid, all of which can be found on O‘ahu (Chapter 1).

I conducted sentinel egg surveys across the island of O‘ahu, the commercial center of Hawaii, to better understand the current diversity and distribution patterns of both native and introduced Lepidopteran egg parasitoid species. Understanding where alien and native egg parasitoids are distributed in Hawai‘i will be essential to understanding the benefits they may confer to agriculture, and the damage they may be causing to native insects, which are largely confined to higher elevations.

Materials and Methods

Study organisms

The eggs of three moth species were used in this study: *Daphnis nerii* (Sphingidae) *Agrius cingulata* (Sphingidae), and *Helicoverpa zea* (Noctuidae). All three species were chosen because they were already established on the island of O‘ahu and are known to be the targets of egg parasitoids (Moore and Miller, 2008). Further, *H. zea* was used because its life cycle has been well documented and it is relatively easy to mass rear (Shorey and Hale, 1965).

Rearing of Daphnis nerii

Caterpillars and eggs of *D. nerii* were collected from oleander, *Nerium oleander* L. (Apocynaceae), found on the University of Hawaii of Manoa campus and vicinity. Caterpillars were fed in groups with fresh clippings of oleander in ventilated containers until their third instar. Caterpillars were then moved outside into rearing cages with oleander cuttings inserted into plastic food storage containers filled with water. When caterpillars turned black and orange in preparation for pupation, they were transferred to 4.6 L plastic buckets filled to a depth of 5 cm with commercial potting soil. Female and male pupae were sorted and put into separate collapsible butterfly cages (38 cm length x 38 cm width x 58 cm height). If females eclosed prior to males, the adult females were placed in new butterfly cages outside to call in wild males. This species readily oviposited on host plants within flight cages. While previous work suggested that these moths can be reared only in large flight cages (Zagorinskii et al. 2013), I found that they would mate both indoors and outdoors in the small collapsible cages described above.

Rearing of Agrius cingulata

Eggs of *A. cingulata* were collected on *Ipomoea tuboides* Otto Degener and Oostroom (Convolvulaceae) found at Kawainui Marsh (Kawai Nui Neighborhood Park, Kailua) and caterpillars were reared on *I. tuboides* in 4.6 L buckets with ventilated tops at a temperature of 24°C. Caterpillars were moved to fresh buckets containing commercial potting soil when they began to wander prior to pupation as described above for *D. nerii*. As with *D. nerii*, the adult moths were placed and mated outdoors in collapsible flight cages containing potted live host plants on elevated benches at 33 cm above the ground. Eggs were collected from host plants and prepared immediately for field deployment.

Rearing of Helicoverpa zea

Eggs of *H. zea* were collected from a corn field in Kunia, in the central part of Oʻahu. Caterpillars were reared on a generalized soy-wheat germ Lepidoptera diet (Frontier Scientific Services) which was poured into 32 mL plastic cups (Dixie item nos. P,C,1, PL1), with 6ml of diet in each cup. The caterpillars were placed in individual cups (Fig. 2.1) and once the caterpillars pupated, the cups were moved into a rearing cage with host plant (corn) as an oviposition substrate. Feeding stations were provided, constructed from 58 mL plastic portion cups (Solo item nos. P200, PL2) filled with a 1:9 mixture of honey and water. A hole was melted in the top of the lids and cotton wicks were inserted into the cups to absorb the honey water for the adults to feed upon. The eggs of this species were collected off of the host plant, the screen walls of rearing cages, and the cotton wicks on feeding stations.

Field sites

I selected eight sites across the island of Oʻahu, at which sentinel egg cards were placed between May 2017 and July 2018 (Fig. 2.2). The sites included the two mountain ranges on Oʻahu: Koʻolau and Waiʻanae. Sites were chosen based on accessibility and elevation to ensure coverage of a broad elevational gradient to evaluate the impact of elevation on species distributions (Table 2.1). Mean annual rainfall was taken from "Rainfall Atlas of Hawaiʻi (Frazier et al. 2016) using the coordinates provided in Table 2.1.

Figure 2.1 Rearing *Helicoverpa zea* in Dixie cups.

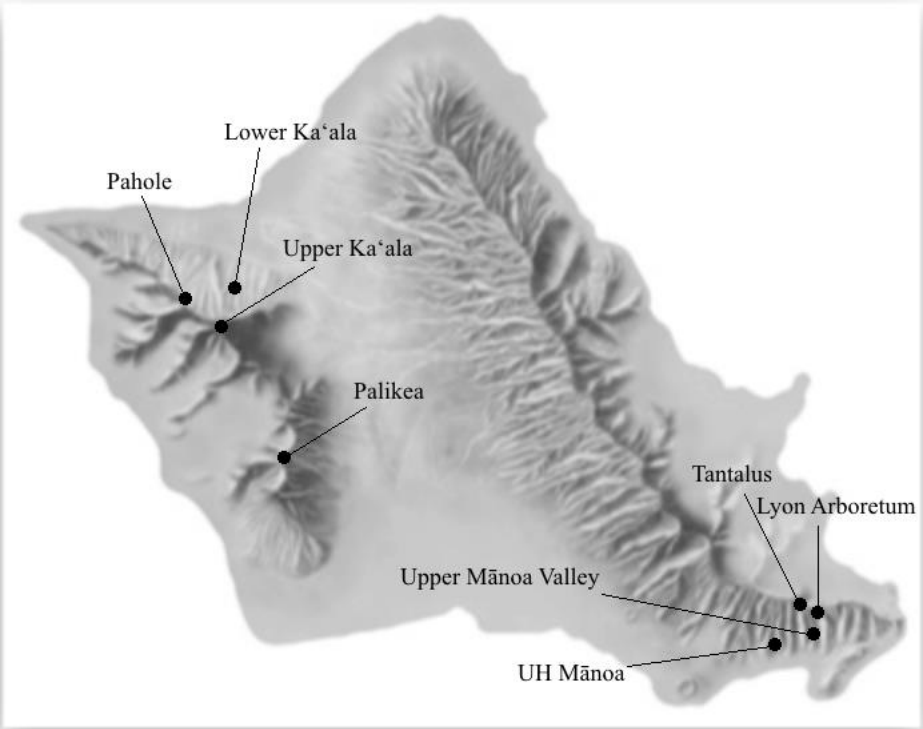

Figure 2.2 Eight study site locations on O‘ahu: UH Mānoa, Tantalus, Upper Mānoa Valley, Lyon Arboretum, Upper Ka‘ala, Lower Ka‘ala, Pahole, and Palikea.

Table 2.1 Study locations and characteristics

Location	Elevation	Vegetation	Mean Annual Rainfall	Lat and Long
UH Mānoa	42 m	Non-Native	999 mm	21.30085, -157.82002
Upper Mānoa Valley	152 m	Some Native	2604 mm	21.316272, -157.80008
Lyon Arboretum	244 m	Some Native	3836 mm	21.33418, -157.80518
Tantalus Cliff Trail	563 m	Some Native	3892 mm	21.338135, -157.811160
Palehua Palikea Ridge Trail	701 m	Native Forest	810.1 mm	21.394371, -158.097735
Pahole Reserve	848 m	Native Forest	1304.2 mm	21.54037, -158.19318
Upper Ka‘ala	914 m	Native Forest	2015 mm	21.516696, -158.142965
Lower Ka‘ala	152 m	Non-Native	2015 mm	21.551293, -158.156898

Experimental design

At each site, 15 plants were selected haphazardly based on accessibility, usually along trails. To analyze the effect of elevation on parasitism rates, two separate trials were done at various locations and across elevations: season-biased and season-unbiased. The season-biased trial included eggs of *D. nerii*, *H. zea*, and *A. cingulata* deployed at different locations over different months. The reason for deploying different species in different months was because the different moth colonies were not synced in their development. The season-unbiased data included eggs from *H. zea* and *D. nerii* deployed in each location within a two-week time frame, allowing direct comparisons of parasitism among sites and between species within similar deployment periods. Only the lower elevation for Ka‘ala was used in the season-unbiased trials due to site restrictions. Each two-week time frame was designated as a sampling event. The season-biased and season-unbiased data were analyzed separately because they were conducted in different ways, with the season-biased dataset having a potential seasonal bias.

Deployment of sentinel eggs

Small 0.5 cm x 0.5 cm squares were cut from yellow stock paper and used as a substrate for sentinel eggs. Non-toxic glue (Elmer’s Glue All®) was applied to each card using an insect pin

and one egg was applied to each card using a small paint brush. Yellow paper was used because other studies have found yellow to be attractive to lepidopteran egg parasitoids, specifically *Trichogramma* (Lobdell et al. 2005; Pacilly et al. 2011). Plants were labeled with yellow flagging tape to help relocate them in the field, and egg cards were glued to leaves with Elmer's Glue All® on separate branches from the branch that was flagged. The number of eggs set out on a given day per site varied by how many eggs were available and ranged from 30 to 120 with a total number of 3,350 deployed over the course of these studies. If eggs could not be deployed on the day they were laid, they were stored for up to 10 days at 10°C to slow development, following Dhillon and Sharma (2007).

Egg exposure

Egg cards were left in the field for a total of three days because caterpillars usually hatch within four to five days in warm climates like Hawaii's. Deployed eggs were collected on the fourth day and placed into 1.5 ml tubes each labeled with a unique identification number. Eggs were kept in vials in the laboratory at 25°C for one month and monitored daily with the fate of each egg recorded as either: parasitized, hatched, predated or unhatched (Table 2.2 and Table 2.3). Eggs on cards that showed significant chewing damage to the yellow card where the egg had been glued were classified as predated. Eggs that hatched in the lab after retrieval were recorded as such. Occasionally eggs were found hatched in the field and these were also listed as hatched, but if the card was blank upon retrieval with no trace of an egg it was recorded as "disappeared". If parasitoids emerged, I recorded the number of individuals emerging from each egg and preserved parasitoid specimens by point mounting or in 90% ethanol. If a parasitoid could not be

identified from its morphology, identification was attempted using genetic methods (see "Parasitoid Identification and DNA analysis protocols").

Table 2.2 Season-biased egg exposures

Location	Species of moth	Total number of eggs deployed	Total number of eggs retrieved	Total number of eggs hatched	Total number of eggs parasitized	Total number of eggs predated
UH Manoa	*Daphnis nerii*	270	213	165 (77%)	19 (9%)	9 (4%)
UH Manoa	*Helicoverpa zea*	30	27	20 (74%)	0	1 (4%)
Upper Manoa Valley	*Daphnis nerii*	60	45	8 (18%)	16 (36%)	2 (4%)
Lyon Arboretum	*Daphnis nerii*	150	147	133 (90%)	6 (4%)	5 (3%)
Lyon Arboretum	*Agrius cingulata*	90	71	33 (46%)	3 (4%)	2 (3%)
Tantalus	*Daphnis nerii*	240	219	200 (91%)	4 (2%)	7 (3%)
Tantalus	*Helicoverpa zea*	90	71	42 (59%)	0	2 (3%)
Tantalus	*Agrius cingulata*	90	81	21 (0.3%)	1 (1%)	2 (2%)
Palikea	*Daphnis nerii*	180	165	85 (52%)	0	0
Palikea	*Helicoverpa zea*	150	102	43 (42%)	2 (2%)	0
Pahole	*Agrius cingulata*	90	76	7 (9%)	0	2 (3%)
Pahole	*Helicoverpa zea*	150	92	31 (34%)	1 (1%)	5 (5%)
Upper Ka'ala	*Daphnis nerii*	120	94	61 (65%)	0	0
Upper Ka'ala	*Helicoverpa zea*	300	168	44 (26%)	0	6 (4%)
Lower Ka'ala	*Helicoverpa zea*	120	71	22 (31%)	0	3 (4%)

Table 2.3 Season-unbiased egg exposures

Location	Species of moth	Number of eggs deployed	Number of eggs retrieved	Total number of eggs hatched	Total number of eggs parasitized	Total number of eggs predated
UH Manoa	*Helicoverpa zea*	75	50	32 (64%)	0	2 (4%)
UH Manoa	*Daphnis nerii*	15	13	12 (92%)	0	0
Upper Mānoa Valley	*Helicoverpa zea*	45	43	17 (40%)	1 (2%)	0
Upper Mānoa Valley	*Daphnis nerii*	15	10	4 (40%)	4 (40%)	0
Lyon Arboretum	*Helicoverpa zea*	75	45	12 (27%)	0	1 (2%)
Lyon Arboretum	*Daphnis nerii*	15	11	6 (55%)	0	0
Tantalus	*Helicoverpa zea*	75	57	22 (39%)	2 (4%)	0
Tantalus	*Daphnis nerii*	15	11	7 (64%)	0	0
Palikea	*Helicoverpa zea*	165	135	24 (18%)	3 (2%)	1 (1%)
Palikea	*Daphnis nerii*	15	14	9 (64%)	3 (21%)	0
Pahole	*Helicoverpa zea*	60	34	6 (18%)	0	0
Lower Ka'ala	*Helicoverpa*	60	36	0	2 (6%)	1 (3%)

Plant choice test

We conducted a small trial to test whether plant species used as a substrate generally had an impact on the frequency of parasitism in sentinel eggs. This trial took place at the "Upper Manoa Valley" location, a residential area that was landscaped with a variety of native and nonnative plants. A total of 122 eggs were deployed for this smaller trial (see Table 2.4). Eggs from two species of moths from two different families were used in this plant choice trial: *D. nerii* (Sphingidae), and *A. odorata* (Noctuidae). Eggs were deployed on eight different plant species (Table 2.5). One plant species: *Paederia foetida* (Rubiaceae) was used as a positive control because it is the local (introduced) host plant of the introduced moth *Macroglossum pyrrhostica,* Butler (Lepidoptera: Sphingidae) which were observed to be heavily parasitized by the same wasp species targeting *Daphnis nerii* in earlier trials (unpublished data): *Ooencyrtus pallidipes*, *Trichogramma chilonis*, and a *Telenomus sp*. Seven plants of *P. foetida* were used to compare parasitism against the seven other plant species at the same location to determine if *P. foetida*

influenced parasitism. There were more eggs deployed on the host plant *P. foetida* than any other plant species as a result of this comparison study (Table 2.5). Parasitism in *H. zea* was very low compared with *Daphnis nerii* in early trials and we wanted to determine if another noctuid, with a larger egg size, suffered greater parasitism due to its ability to house larger parasitoid species. Two to four eggs of both *D. nerii* and *A. odorata* were deployed, depending on egg availability over the course of the study. The eggs of both species were not deployed at the same time. A total of 60 eggs from *D. nerii* were deployed in July 2017 and a total of 90 eggs of *A. odorata* were deployed in April and May 2018. Each egg retrieved was counted as a data point, and a total of 122 eggs with *D. nerii* having a sample size of 45 and *A. odorata* with a sample size of 77. Data was analyzed using mixed model ANOVA to determine if parasitism rates on the known sphingid host plant *P. foetida* differed from parasitism rates on other host plants (see data analysis section). All other data regarding parasitoid species found during this test are catalogued in each trial under "Upper Mānoa Valley".

Table 2.4 Summary of eggs deployed by moth species in upper mānoa valley plant choice test

Species of moth	Total number of eggs deployed	Total number of eggs retrieved	Total number of eggs hatched	Total number of eggs parasitized	Total number of eggs predated
Daphnis nerii	60	45	8 (18%)	16 (35%)	0
Ascalapha odorata	90	77	38 (49%)	19 (25%)	0

Table 2.5 Egg parasitism on plant species categorized by moth species

Plant Species	Species of Moth	Total number of eggs deployed	Total Number of Eggs Retrieved	Total number of eggs parasitized
Rauvolfia sandwicensis	*Daphnis nerii*	4	3	3
Rauvolfia sandwicensis	*Ascalapha odorata*	6	5	4
Hibiscus kokio	*Daphnis nerii*	4	3	0
Hibiscus kokio	*Ascalapha odorata*	6	6	1
Pittosporum glabrum	*Daphnis nerii*	4	4	1
Pittosporum glabrum	*Ascalapha odorata*	6	6	1
Hibiscus arnottianus	*Daphnis nerii*	4	3	0
Hibiscus arnottianus	*Ascalapha odorata*	6	6	0
Pipturus albidus	*Daphnis nerii*	4	4	1
Pipturus albidus	*Ascalapha odorata*	6	6	0
Citrus	*Daphnis nerii*	4	3	0
Citrus	*Ascalapha odorata*	6	6	1
Acacia koa	*Daphnis nerii*	4	3	0
Acacia koa	*Ascalapha odorata*	6	5	1
Paederia foetida	*Daphnis nerii*	30	22	10
Paederia foetida	*Ascalapha odorata*	45	37	11

Parasitoid Identification and DNA analysis protocols

Parasitoids were identified using morphological features. Specimens of *Ooencyrtus* (Encyrtidae), and the unknown eupelmid were mounted on points and then identified to family following Huber and Goulet (1993) and by comparison with specimens in the University of Hawaii Insect Museum. *Trichogramma* were slide mounted and identified using a key for *Trichogramma* of Hawaii (Oatman et al. 1982). Specimens were also stored in 95% ethanol for DNA extractions. DNA was extracted from individual parasitoids by crushing them in 1.5 ml tubes and following protocols in the Qiagen DNeasy Blood and Tissue kit. Polymerase Chain Reaction was used to amplify sequences from the Internal Transcribed Spacer 2 and Cytochrome c Oxydase 1 gene regions. The ITS2 primers used were ITS2-F (5'-TGTGAACTGCAGGACACATG-3') (Santos at al. 2015) and ITS2-trich (5'-GTCTTGCCTGCTCTGAG-3') (Stouthamer et al. 1999). The CO1 primers used were LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer at el. 1994). These regions were targeted because they are the among the most widely used for identification of Hymenoptera using DNA barcoding. Samples were then sent to a commercial sequencing service (Eurofins, Luxembourg) for Sanger sequencing. Sequence data was analyzed using the Geneious software package (Prime 2019) to align forward and reverse sequences, map to a reference sequence, and check for stop codons. Sequences were then submitted to GenBank and assigned reference numbers (Table 2.6).

Table 2.6 GenBank reference numbers and primers

Parasitoid	Primer Used	Annealing Temperature	Gene targeted	GenBank Reference numbers
Ooencyrtus pallidipes	ITS2F/ITS2trich	55°C	ITS2	MH780872-MH780879
Trichogramma chilonis	ITS2F/ITS2trich	55°C	ITS2	MH780863-MH780866
Trichogramma chilonis	LepF1/LepR1	53°C	CO1	MH801180-MH801183
Unknown eupelmid	ITS2F/ITS2trich	55°C	ITS2	MN614145

Data analyses

All data in this study were analyzed using JMP PRO statistical software. The data in this study were divided into two categories: season-unbiased and season-biased and these data sets were analyzed separately and then compared. A mixed model ANOVA was used to analyze relationships between the response variable total percentage of eggs parasitized and the explanatory variables elevation, moth species, and month of deployment. A mixed model ANOVA was used because the JMP software's mixed model uses maximum likelihood estimates of means which makes it less prone to issues with inequality in variances among treatments. A mixed model ANOVA test also avoids biases due to missing data points because it incorporates every data point with no deletions and, as a result, the missing egg data do not impact the results from this data analysis (Krueger, 2004). The categorical explanatory variables included: moth species, elevation, and month of release. Total percentage of parasitism was used as the response, and the observational units of this test were the total number of eggs retrieved from each moth species at a given location in a given month. A Fisher's exact test was used for contingency table analysis of parasitism by elevation and by moth species. Due to the small sampling size within each species of moth and the comparison of two nominal variables, a Fisher's exact test was

used. Standard Least Squares ANOVA was used to analyze the association between parasitism of sentinel eggs across elevations for the combined data from all moth species.

Parasitoids found

All parasitoids were identified by morphology using a key to families of Hymenoptera by Huber & Goulet (1993). For DNA amplification products, I tried to identify each parasitoid through a BLAST (Basic Local Alignment Search Tool) search (Madden 2002), but most of the matches were not similar enough to confirm a species-level identification. The closest sequence to the *Ooencyrtus* vouchers came from the ITS2 region with an undescribed *Ooencyrtus* sp. at 96.84%. The CO1 gene region sequences from my collected *Trichogramma* gave a 100.00% match to *Trichogramma chilonis*. An unknown wasp was collected six times in sentinel sphingid eggs (*D. nerii*) and no similar vouchers were found in the Bishop Museum or the University of Hawai‘i. This wasp was keyed out to be in the family Eupelmidae, but did not have any close DNA matches from the ITS2 gene region, with wasps from both Pteromalidae and Encyrtidae matching at 85%. The CO1 sequence (115 bp) was not long enough for a successful identification. Sequences were submitted to GenBank; reference numbers can be found with their respective primers in Table 2.6.

None of the three species of parasitoids reared were native to the islands. Only *T. chilonis* was found in native vegetation and at a higher elevation (701 m). This *Trichogramma* species was introduced as a biological control agent for the Asiatic rice borer in 1929 (Funasaki et al. 1988). *Trichogramma chilonis* was also reared at lower elevations from sentinel eggs in UH Mānoa and Upper Mānoa Valley.

The most abundant parasitoid species collected was *Ooencyrtus pallidipes* (Ashmead) (Encyrtidae) which was purposefully introduced as a biocontrol agent for the banana skipper *Erionota thrax,* Hübner (Lepidoptera: Hesperiidae) in 1973 (Mau et al., 1980), but has spread to a wide range of other Lepidoptera. A significant difference in the variety of parasitoid species was found between lepidopteran hosts (Fisher's exact test $P < 0.0013$). *Trichogramma* was the only parasitoid to target *H. zea,* while the sphingid eggs were both targeted by multiple parasitoid species.

Plant choice test

Two least squares contrast tests were used to examine any association between *P. foetida* and parasitism, one for each moth species. The response variable in this study was total percentage of parasitism and the explanatory variable was the plant used. The contrast was set up so that total percent parasitism from *P. foetida* was compared to the total percent parasitism on the six other plants used. Two separate analyses were used because each species was deployed in a separate month and did not share a common timeframe. Separate deployments were necessary because it was difficult to maintain large numbers of eggs from different species of moths at the same time, therefore species were used sequentially for trials. A mixed ANOVA model test was used to determine if moth species had an effect on parasitism with the response variable as total percent parasitism and the explanatory variable being moth used. This was a separate analysis because this test included both moths (N=122).

Results

Out of the 3,350 deployed, 1,988 sentinel egg cards were retrieved and only 63 eggs were parasitized (3.1% of the eggs retrieved); 35 of the total eggs deployed were predated (1.7%), 1054 (53%) successfully hatched, and 1,362 eggs disappeared (40.6%). The percentage of eggs parasitized at each location in the season-biased data is listed in Table 2.7 and the percentage of eggs parasitized in the season-unbiased data is listed in Table 2.8.

Plant choice test

No significant association was found in both mixed model ANOVAs between plant species and parasitism for either *A. odorata* ($F_{1,41} = 0.4783$, $P = 0.4931$) or *D. nerii* ($F_{1,37} = 2.7335$, $P = 0.1067$). We did not find an effect of moth species on parasitism ($F_{1,86} = 0.9339$, $P = 0.3366$).

Season-biased

2,640 eggs from three species of moths (*H. zea*, *D. nerii*, and *A. cingulata*) were deployed in these trials. Out of the 1,599 eggs retrieved: *A. cingulata* (n = 228), *D. nerii* (n = 883), and *H. zea* (n = 460), only 52 eggs were parasitized (3.25%). Before analyzing the association between elevation and parasitism, a least squares ANOVA was done to determine if moth species had an effect on total percentage of parasitism through mix modeling. Moth species was treated as a categorical explanatory variable while total percentage of parasitism was a continuous response variable. The units in this study were the total number of eggs retrieved from a species in each sampling event. The sample size varied for each species of moth depending on available eggs. The results of this least squares ANOVA test revealed that there was not a significant relationship between the moth species used and parasitism ($F_{2,46}=1.99$, $P = 0.1482$; Fig.2.3). A

contingency table was used to make a mosaic plot to graphically display parasitism attributable to various parasitoid species over localities (Fig. 2.4) and a Fisher's exact test was employed to test for differences in the wasp species responsible for parasitism at different locations. A significant difference was found ($P < 0.0001$) in the proportion of parasitism from different parasitoid species among localities. A one-way ANOVA comparing mean percentage of parasitism over different elevations by species revealed a significant effect of elevation on parasitism of *D. nerii* by parasitoids, with greater parasitism occurring at low elevations ($F_{5,30} = 5.5$, $P = 0.0015$). However, *Helicoverpa zea* did not show a significant difference in parasitism incurred at different elevations ($F_{5,12} = 1.2$, $P = 0.3951$). *A. cingulata* was not deployed at all sites and therefore we did not test for a relationship between elevation and parasitism for this species. In total, three species of parasitoids emerged from the exposed eggs (Table 2.9). To analyze frequency of parasitoid species across sites, two contingency tables were made: frequency of parasitoid species collected from the sentinel sphingid eggs *(D. nerii* and *A. cingulata*); and frequency of parasitoids collected from *H. zea* (Noctuidae) eggs. Frequency of parasitism was found to be significantly greater in the sentinel sphingid eggs (Fisher's exact, $P < 0.0001$) as seen by the mosaic plot (Fig. 2.5).

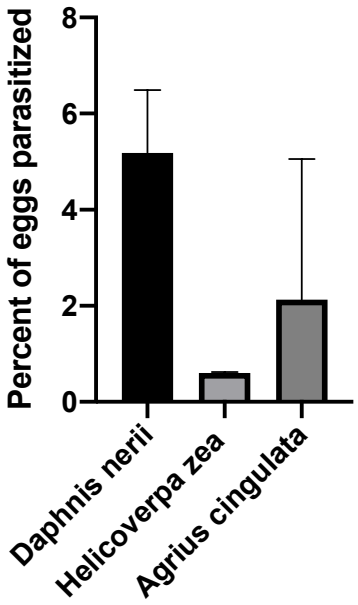

Figure 2.3 Mean (±SEM) showing the difference in mean parasitism by host egg species of Lepidoptera.

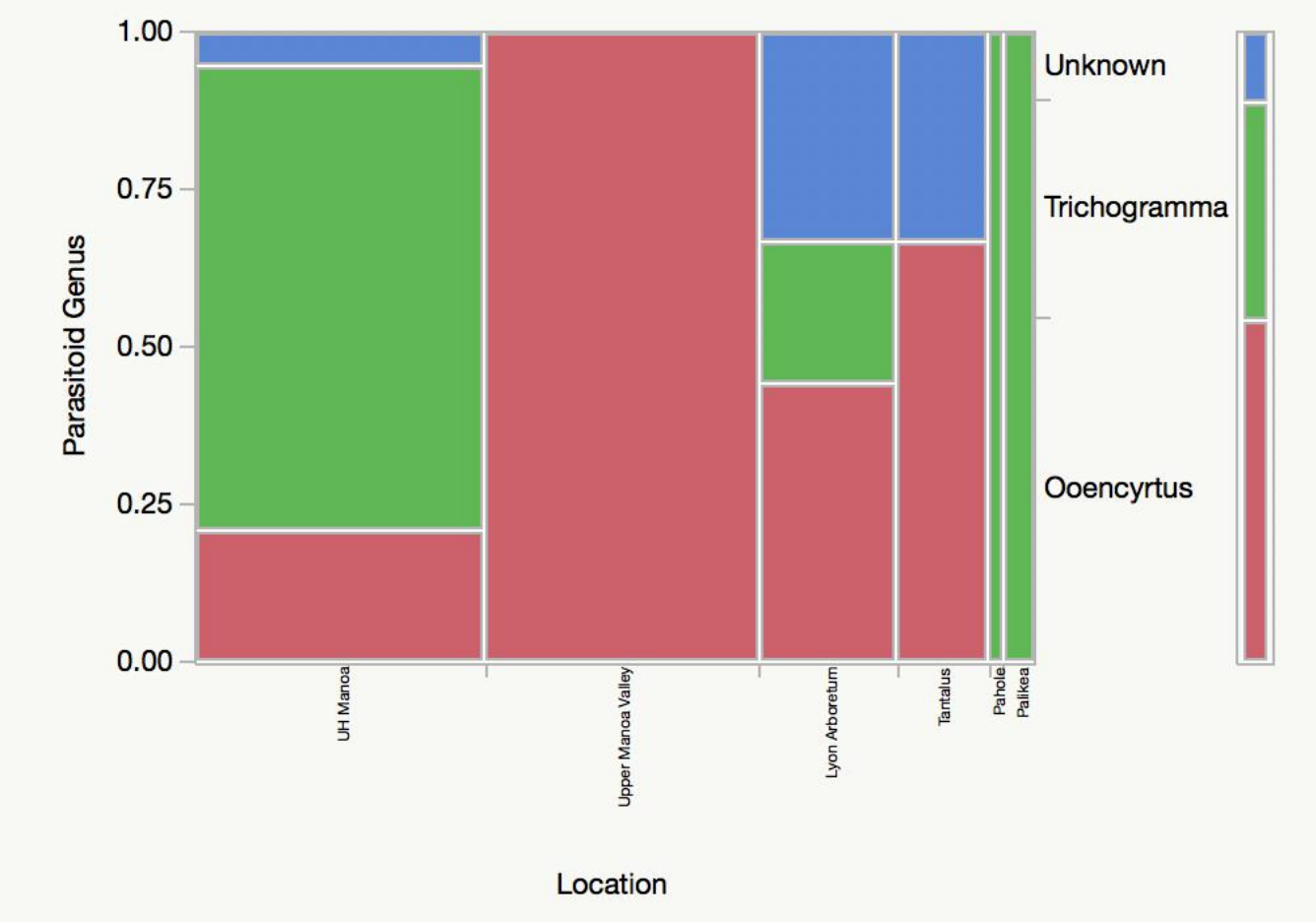

Figure 2.4 Mosaic plot of parasitoid species in three Hymenoptera families found in each location

This figure shows the proportional abundances of parasitoid species collected at a location, with a significant difference found in occurrence of parasitoid species between localities. The proportion of parasitoid species at each site is seen on the y-axis while the x-axis is proportional to the total parasitism rate at each site.

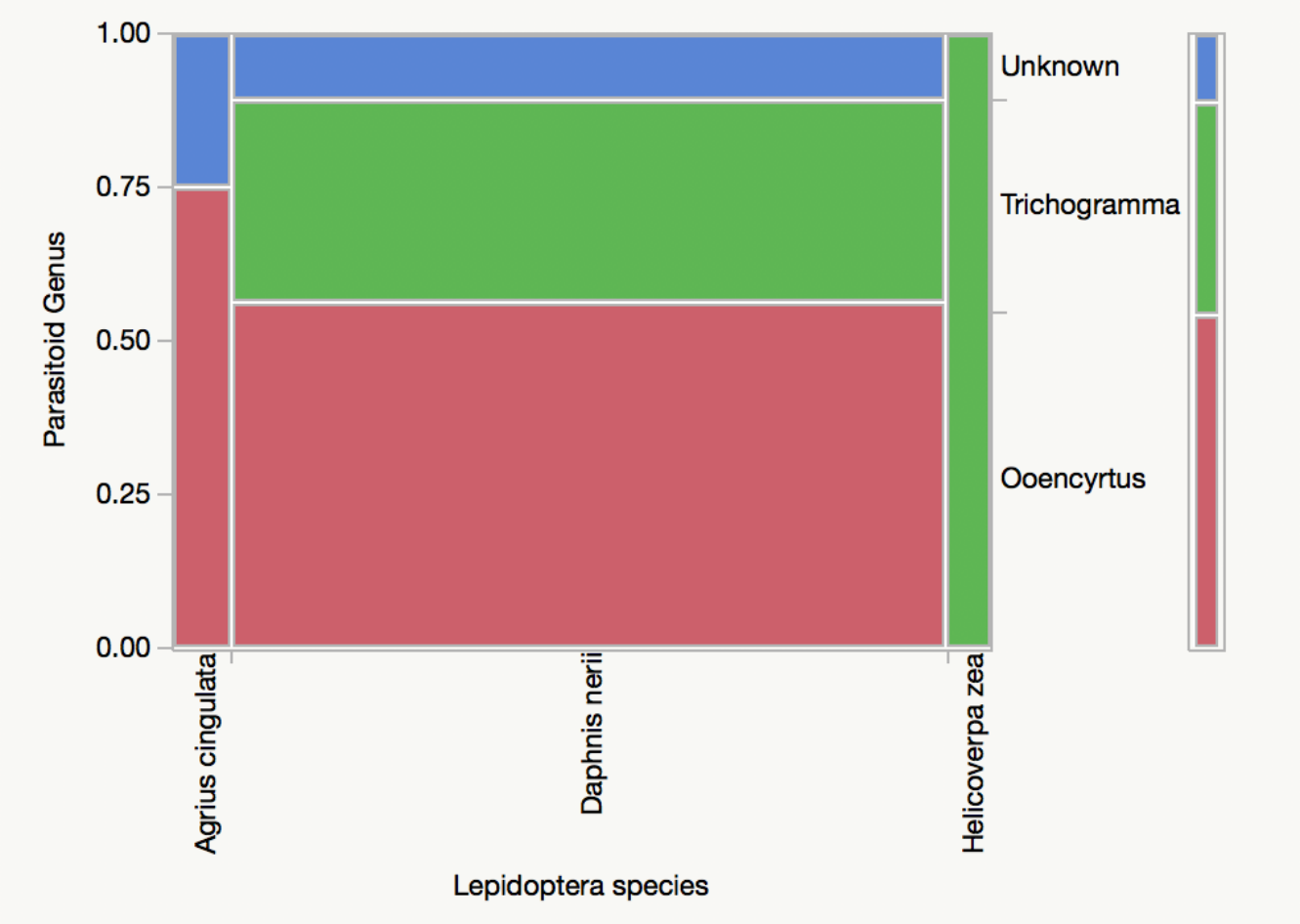

Figure 2.5 Mosaic plot of parasitoid species found in different lepidopteran hosts

This mosaic plot depicts the proportion (y-axis) of parasitoid species reared from each sentinel host. The x-axis is proportional to the total number of parasitoids reared from each species. Both sphingid hosts had a significantly greater number of parasitoid species reared out than the noctuid *Helicoverpa zea*.

Season-unbiased

Out of the 710 eggs of *H. zea* and *D. nerii* deployed to examine the impact of time of year on parasitism, 459 were retrieved. Out of the 459 eggs retrieved, only 12 were parasitized (2.6%).

The least squares ANOVA test to determine if moth species had an effect on parasitism showed no significant difference in parasitism between the two moth species ($F_{1,23} = 2.09$, $P = 0.1614$). No significant relationship was found between total percentage of eggs parasitized and elevation ($F_{6,18} = 1.02$, $P = 0.4443$) or month of deployment ($F_{1,6} = 0.19$, $P = 0.6671$). There was also no difference in the parasitoid species found from the season-biased data.

Table 2.7 Parasitism by location in season-biased dataset

Location	Elevation	Total number of eggs retrieved	Total number of eggs parasitized	Percentage of eggs parasitized
UH Manoa	42 m	240	19	8 %
Upper Manoa Valley	152 m	45	16	36 %
Lyon Arboretum	244 m	218	9	4.13 %
Tantalus	563 m	371	5	1.34 %
Pahole	701 m	168	1	0.60 %
Palikea	848 m	267	2	0.75 %
Upper Ka‘ala	914 m	191	0	0.00 %
Lower Ka‘ala	152 m	71	0	0.00%

Table 2.8 Parasitism by location in season-unbiased dataset

Location	Elevation	Total number of eggs retrieved	Total number of eggs parasitized	Percentage of eggs parasitized
UH Mānoa	42 m	63	0	0.00%
Upper Mānoa Valley	152 m	53	5	19.38 %
Lyon Arboretum	244 m	56	0	9.43 %
Tantalus	563 m	68	2	2.94 %
Pahole	701 m	34	0	0.00 %
Palikea	848 m	149	2	1.34 %
Lower Ka’ala	152 m	36	2	6.00 %

Table 2.9 Parasitoid Species retrieved and sentinel host

Parasitoid Family	Parasitoid Species	Sentinel Egg moth Species	Location
Encyrtidae	*Ooencyrtus pallidipes*	*Daphnis nerii & Agrius cingulata*	Tantalus, Lyon Arboretum, UH Mānoa, Upper Mānoa Valley.
Eupelmidae	Unknown species	*Daphnis nerii & Agrius cingulata*	Tantalus, Lyon Arboretum, UH Mānoa.
Trichogrammatidae	*Trichogramma chilonis*	*Daphnis nerii & Helicoverpa zea*	Palikea, Ka'ala, Upper Mānoa Valley, Tantalus.

Discussion

This study is the first on Oahu to survey for lepidopteran egg parasitoids using sentinel eggs across an elevational gradient. The objectives of this study were to develop a species list of egg parasitoids on Oahu, to document unrecorded species, and to examine the impact of moth host species and elevation on patterns of parasitoid diversity. This information is important for future studies involving egg parasitism and is useful for future biological control projects, both for control of pest species and to ensure that parasitoids already present in Hawaii don't inhibit the use of Lepidoptera for biological control of weeds.

Parasitoids found

Only three parasitoids were found in the duration of this study: *Ooencyrtus pallidipes*, *Trichogramma chilonis*, and an unknown eupelmid. There are currently 17 known Lepidopteran egg parasitoids within the Hawaiian Islands (see Chapter 1) and the majority of these parasitoids have been documented on O'ahu (Nishida 2002). Of these 17 egg parasitoids only 4 are

documented to be non Trichogrammatidae. During this study I was able to collect two non-trichogrammatid egg parasitoids of Lepidoptera and one trichogrammatid. *T. chilonis* is known for being the primary egg parasitoid of *H. zea* in the Hawaiian islands and does a good job suppressing populations in the summer months (Vargas & Nishida, 1982) and *O. pallidipes* has been successful in the suppression of banana skipper populations in Hawai‘i (Mau et al. 1980). I found no literature on Lepidopteran egg parasitoids in the family Eupelmidae from the Hawaiian Islands. As a result the unknown eupelmid might represent a new host record or a new species to the islands. Using sentinel eggs from a small handful of moth species may have found one unrecorded species but it was not a successful approach for detecting a variety of new lepidopteran egg parasitoid species nor was it a good representation of the parasitism seen in wild moths (see Chapter 3).

Elevation and parasitism

Parasitism was found to be significantly lower at higher elevations in the season-biased dataset, but we found no significant effect of elevation in the season-unbiased data. These results imply that there may be a seasonal effect on egg parasitism but no significance was found in parasitism between the months surveyed. It’s very possible the lack of significance between elevations in the season unbiased data came from having *H. zea* as the main source of eggs. As stated previously, *Trichogramma* were the only parasitoids to target *H. zea* in this study, and they were also the only parasitoid found in this study at high elevations. It is possible that, because this *Trichogramma* occurs at both low and high elevations, there was no significant association found between parasitism and elevation for *H. zea*. However, another possible explanation for no significant association is that the percentage of parasitism by *H. zea* was too low at all elevations

to have enough statistical power to detect a difference in parasitism between elevations, if one were present.

The significantly greater percentage of parasitism found at lower elevations in the season-biased data might be explained by the difference in fauna and flora between elevations. There is a much greater diversity of introduced species of both plants and insects at lower elevations in Hawai‘i (Smith, 1985) which could explain why a majority of the parasitism of nonnative moth eggs by these nonnative egg parasitoids occurred at lower elevations. A similar study was conducted on Hawai‘i Island surveying the distributions of larval parasitoids over an elevational gradient of various sites, and this study also found both a greater diversity and greater parasitism in invasive parasitoids at lower elevations (Peck et al. 2008). The greater number of Sphingidae eggs used in the season-biased data set cannot fully explain the significantly higher parasitism at lower elevations in this data set because the *Trichogramma* species found at higher elevations accounted for the majority of parasitism in sphingid eggs (73.68%) at the lowest elevation (UH Manoa).

Future studies

Our results showed low levels of parasitism in sentinel eggs, but this does not mean there are low rates of parasitism in wild populations of Lepidoptera on O‘ahu. The low parasitism we found may be the result of a lack of natural interactions between host plants and the eggs we placed on them, since Lepidoptera would normally cause larval feeding or oviposition damage on the plant, releasing kairomones that might attract egg parasitoids (Colazza, 2004). Additionally, a majority of the plants used as substrates in this study are not hosts of the lab-reared Lepidoptera due to practical limitations regarding the use and rearing of native Lepidoptera for such an experiment.

This is especially true for the higher elevation locations such as Ka‘ala where a majority of the flora is endemic, but the eggs deployed were nonnative. Egg parasitoids rely on many chemical factors to find eggs such as moth scale kairomones (Delury et al., 1999; Smith, 1996), volatile emissions from oviposition (Colazza, 2004), sex pheromones (Peri et al., 2006), and residual chemicals from adults on host plants (Delury et al., 1999; Peri et al., 2006).

One reason for examining parasitism over an elevation gradient is to document the invasion of relatively intact native ecosystems by non-native wasps. O‘ahu is highly developed and most of its native vegetation has been restricted to fragments at higher elevations. Based on the data collected, there are fewer species of egg parasitoids at higher elevations on Oahu, but the single species of parasitoid found at higher elevations was introduced as a biological control agent. A study of parasitism of Lepidoptera larvae by Peck et al. (2007) on Hawai‘i Island found an overall dominance of larval alien parasitoids at both high and low elevations (Peck et al., 2008). However, a different study found that the endemic moth genus *Hyposmocoma,* Butler (Cosmopterigidae) only yielded native larval parasitoids (Henneman & Memmott, 2001). It is possible that because the only eggs used in this sentinel egg parasitoid study were non-native, we did not observe parasitism by native egg parasitoids that could be inhabiting higher elevations of O‘ahu.

Chapter 3. Surveys of Parasitism in wild collected and sentinel Lepidopteran eggs on Oʻahu

Abstract

Hawaiʻi is known for its high levels of endemism among insects and plants in particular, but it also has some of the highest numbers of introduced species of arthropods in the world. Many of these introduced arthropods are parasitoids used to control populations of adventive insect pests. Egg parasitoids in particular are often used to target pest populations of Lepidoptera. Egg parasitoids are typically minute insects that are not readily detected during quarantine inspections, or during conventional insect collecting efforts. There have been few systematic efforts to detect and document newly adventive egg parasitoid species in Hawaiʻi, or to measure their potential impacts on native lepidopteran populations. I surveyed Oʻahu for unrecorded egg parasitoids by collecting eggs from wild populations of Lepidoptera on Oʻahu. To assess the effectiveness of sentinel eggs, and to detect egg parasitoid diversity, I also compared parasitism and parasitoid species between wild eggs and deployed sentinel eggs. In total 540 eggs were collected in the wild of which 319 (59.1%) were parasitized and 2,030 sentinel eggs were retrieved of which only 3.1% were parasitized; simple correlation coefficients were taken to compare percent parasitism, proximity to ports, and elevation between wild collected and sentinel eggs. My results reveal that parasitoid species differ by location, and wild collected eggs are parasitized at a higher rate than sentinel eggs, suggesting that such sentinels may not be effective measures of parasitoid impacts or diversity. A negative correlation was found for percent parasitism and proximity to ports in wild collected eggs, and both sentinel and wild

collected eggs revealed a mild negative correlation between percent parasitism and elevation with parasitism decreasing at higher elevations.

Introduction

The state of Hawai‘i has more nonnative arthropods than any other state in the USA, and about 22% of the purposefully introduced species (predators and parasitoids) are known to be generalists in their host use (Messing and Wright 2006). It is estimated that nearly 20 new species of arthropod reach the islands each year aided by human activities such as movement of agricultural goods and horticultural materials (Beardsley 1979). Most biological control introductions in Hawai‘i were intended to function as classical biological control agents: natural enemies of established pest species introduced to suppress pest populations. Hawai‘i has been a proving ground for classical biological control for over 100 years, with 679 species introduced for insect and weed biocontrol between 1890 and 1985 alone (Funasaki et al. 1988). This influx of purposefully introduced species, and some papers criticizing classical biological control in Hawai‘i, has caused an erosion of public support for biological control because Hawai‘i has remarkable levels of endemism, and the introduction of predatory and parasitic insects with possible non-target impacts has been considered an unacceptable practice (Howarth 1991; Johnson et al. 2005). It has been suggested that classical biological control agents are responsible for both population reductions and extinctions in native Hawaiian Lepidoptera, and that introduced parasitoids had the worst impact (Zimmerman 1948; Howarth 1983; Funasaki et al. 1988; Kaufman and Wright 2010).

Parasitoids are usually wasps and flies that develop within their hosts, killing their prey as a result. This makes parasitoids prime candidates for biological control programs in pest

management. There are three well known broad guilds of parasitoids; those that target the eggs, larval, or pupal stages of their host. Some parasitoids, such as some braconid wasps, have plasticity in their host use and are known to attack different life stages depending on what is available. Other parasitoids are generalists in their host use, there are also some species which are oligo- or monophagous. Egg parasitoids are among the smallest and least conspicuous insects, but can be important in biological control, especially the superfamilies Platygastroidea and Chalcidoidea. To date there have been a limited number of studies examining egg parasitism of native insects in Hawai‘i (King et al. 2010; Johnson et al. 2005). The majority of parasitoid research in Hawai‘i has addressed larval and pupal parasitism (Henneman and Memmott 2001; Kaufman and Wright 2010; Duan and Messing 1997). This is probably because egg parasitoids are generally not as successful in suppressing pest populations as species that attack later life stages (Mills 2009; Öztemiz et al. 2017).

To better understand the current diversity of Lepidopteran egg parasitoids and their distribution on O‘ahu, I conducted surveys for Lepidopteran eggs across the island. I compared the results from this study with a similar study focused on sentinel egg trials (See Chapter 2) to determine the best approach for collecting egg parasitoids and detecting new adventive species on O‘ahu.

Materials and Methods

Wild egg collections

Eggs from various species of Lepidoptera were collected from March 2017 to May 2018. Eggs were collected from the leaves of their host plants in different locations, and then placed individually in 1.5ml microcentrifuge tubes labeled with the name of the location (Table. 3.1)

and date collected. Eggs were monitored in the 1.5ml tubes after collection for signs of parasitism and the fate of each egg was categorized as parasitized, caterpillar hatched or dead (unknown causes). A total of 540 eggs were collected over the course of this study. The species of Lepidoptera eggs, plants, and egg parasitoids found are shown in Table 3.3.

Table 3.1 Summary of locations, vegetation and Lepidoptera species collected

Location	Elevations	Vegetation	Closest Proximity to Ports	Species of Lepidoptera eggs collected from Site
UH Mānoa	42m	Non-Native	10 km	*Daphnis nerii*, *Danaus plexippus*
Upper Mānoa Valley	152m	Some Native	14 km	*Macroglossum pyrrhosticta*
Lyon Arboretum	244m	Some Native	16 km	*Macroglossum pyrrhosticta*
Tantalus Cliff Trail	563m	Some Native	16 km	*Macroglossum pyrrhosticta*
Palehua Palikea Ridge Trail	701m	Native Forest	35 km	*Hyles calida*
Waimanalo	9m	Non-Native	42 km	*Lampides boeticus*
Kawainui Marsh, Kailua	5m	Non-Native	54 km	*Macroglossum pyrrhosticta*, *Agrius cingulata*

Sentinel egg deployment

Eggs of lab reared *Daphnis nerii,* Linnaeus (Lepidoptera: Sphingidae), *Helicoverpa zea*, Boddie (Lepidoptera: Noctuidae) and *Agrius cingulata,* Fabricius (Lepidoptera: Sphingidae) (see Chapter 2 for rearing methods) were deployed in the field at seven sites on O‘ahu (Table. 3.1). Eggs of lab reared Lepidoptera were glued on small 0.5cm x 0.5cm squares from yellow stock paper (one egg per square) using Elmer’s Glue All®. Insect pins were used to spread the glue on each card and individual eggs were placed on the cards using a fine paint brush. Yellow cards were used because previous work has demonstrated that yellow is an attractive color for egg parasitoids, specifically *Trichogramma* spp. (Pacilly et al. 2011; Lobdell et al. 2005). At each site I used 15 plants haphazardly selected along trails based on accessibility, upon which sentinel eggs were glued on to the underside of leaves with Elmer’s Glue All®. Eggs were exposed at the locality for three days before their collection. Three days was chosen because caterpillars were observed to hatch around day 5, especially during warmer weather.

Parasitoid Identification and DNA analysis protocols

Parasitoids were identified using morphological characters and dichotomous keys, and DNA barcodes from the Cytochrome Oxydase 1 (COI) and Internal Transcribed Spacer 2 (ITS2) gene regions. Specimens of *Ooencyrtus* spp. (Encyrtidae) were point mounted and identified to family level using keys by Huber and Goulet (1993) and by comparing our specimens with those found in the University of Hawai‘i Insect Museum. *Trichogramma* spp. were slide mounted and identified using a key for *Trichogramma* of Hawai‘i (Oatman et al. 1982). Parasitoids were also stored in 95% ethanol for DNA extractions. The parasitoids stored for DNA were microscopically photographed for vouchers, and then individuals were crushed in 1.5ml tubes for extraction using the Qiagen DNeasy Blood and Tissue kit®. I then used Polymerase Chain Reaction to amplify sequences from ITS2 and CO1 gene regions. The ITS2 primer set used was ITS2-F (5’-TGTGAACTGCAGGACACATG-3’) (Santos at al., 2015) and ITS2-trich (5’-GTCTTGCCTGCTCTGAG-3’) (Stouthamer et a., 1999). The CO1 primer set used was LCO1490 (5’-GGTCAACAAATCATAAAGATATTG-3’) and HCO2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer et al. 1994). These regions were targeted because they are among the most widely used for taxonomic identification of insects, and some were specifically developed for egg parasitoid identification. Samples were then sent to a commercial sequencing service (Eurofins, Genomics) for Sanger Sequencing. Sequences were concatenated using the Geneious software package (Prime 2019) to align forward and reverse sequences as well as map with a reference sequence and check for stop codons. Sequences were compared to a global database of sequences using BLAST (Basic Local Alignment Search Tool) for identification and then submitted to GenBank for reference numbers (Table 3.2).

Table 3.2 GenBank reference numbers and primers

Parasitoid	Primer Used	Annealing Temperature	Gene targeted	GenBank Reference numbers
Ooencyrtus pallidipes	ITS2F/ITS2trich	55°C	ITS2	MH780872-MH780879
Trichogramma chilonis	ITS2F/ITS2trich	55°C	ITS2	MH780863-MH780866
Telenomus sp.	ITS2F/ITS2trich	55°C	ITS2	MH780867-MH780871
Trichogramma chilonis	LepF1/LepR1	53°C	CO1	MH801180-MH801183
Unknown eupelmid	ITS2F/ITS2trich	55°C	ITS2	MN614145

Statistical analyses

A least squares ANOVA test was used to compare parasitism levels in different locations, testing the null hypothesis that percentage parasitism would be equal in all sampled localities. Simple correlation analyses were done to look at the relationship between percent parasitism and both proximity to ports and elevation in wild and sentinel eggs. A contingency table was made to analyze the difference in proportions of parasitoid species found at each location, and a Fisher's exact test was used to test for an association between frequency of parasitoid species and location. Fisher's Exact Test was also used to analyze the association between Lepidopteran species and parasitism due to uneven sampling sizes for species, and small sample sizes for both frequency of parasitoid species by location, and number of wild Lepidopteran eggs collected at a location. Fisher's Exact Test was appropriate for this data set because it does not rely on approximation such as the chi-square test, but rather uses hypergeometric distributions for exact calculations, which are needed for both small and unequal sample sizes as reflected in our dataset. Proportion of parasitism by location was calculated by dividing the total number of eggs parasitized by the total number of eggs collected for each location. Simple correlation

coefficients were taken for both the wild and sentinel eggs to compare percent parasitism between proximity to port and elevation in the shared locations.

Results

In total I collected 540 eggs from the wild and 319 of these were parasitized (59.1%). The eggs collected from two species of butterflies (150) had no parasitism. Three genera of parasitoids: *Ooencyrtus* (Encyrtidae), *Telenomus* (Scelionidae) and *Trichogramma* (Trichogrammatidae) were collected in the wild egg surveys and the percentage of parasitism varied by genus (Table 3.4). Parasitism also varied by location (Table 3.5) with the highest parasitism at the Upper Mānoa Valley sampling site. No Trichogrammatidae were retrieved from Tantalus while *Ooencyrtus* and *Telenomus* were retrieved at every location except UH Mānoa.

A significant difference was found in the frequency of parasitoid species between locations ($P < 0.0001$) with *Ooencyrtus* the most prevalent except on Tantalus and UH Mānoa (Fig. 1). Parasitism varied significantly by location ($F_{6,15} = 5.47$, $P = 0.0035$). There were significant differences in parasitism among Lepidoptera species ($F_{4,17} = 13.76$, $P < 0.0001$) with *M. pyrrhostica* accruing the highest levels of parasitism.

Table 3.3 Summary and fate of wild eggs collected

Location	Plant	Lepidoptera Species	# of eggs collected	# of eggs parasitized	# of eggs that died	# of eggs that hatched	Total% parasitized.
Palikea	*Kadua affinis*	*Hyles calida*	2	0	0	2	0
Lyon Arboretum	*Paederia foetida*	*Macroglossum pyrrhosticta*	121	95	2	24	79%
Tantalus	*Paederia foetida*	*Macroglossum pyrrhosticta*	88	61	8	21	69%
Upper Mānoa Valley	*Paederia foetida*	*Macroglossum pyrrhosticta*	168	151	4	12	90%
Kawainui Marsh, Kailua	*Paederia foetida*	*Macroglossum pyrrhosticta*	1	1	0	0	100%
Kawainui Marsh, Kailua	*Convulvulacea*	*Agrius cingulate*	6	0	0	6	0%
UH Mānoa	*Calatropis gigantea*	*Danaus plexippus*	17	0	6	11	0%
UH Mānoa	*Oleander*	*Daphnis nerii*	15	11	0	4	73%
UH Mānoa	*Adenium*	*Daphnis nerii*	2	2	0	0	100%
Waimanalo	*Crotalaria juncea*	*Lampides boeticus*	133	0	17	116	0%

Table 3.4 Percentage of parasitism by each genus from wild caught eggs, across all Lepidoptera species surveyed (N = 319)

Parasitoid Genera	Percentage of Parasitism
Ooencyrtus	32%
Telenomus	37%
Trichogramma	29%

Table 3.5 Comparison between parasitism in sentinel and wild eggs at shared localities

	Wild Eggs		Sentinel Eggs	
Location	**Lepidoptera spp.**	**Percentage of Parasitism**	**Lepidoptera spp.**	**Percentage of Parasitism**
Upper Mānoa Valley	*Macroglossum pyrrhosticta*	90%	*Daphnis nerii*, *Helicoverpa zea*	21%
Lyon Arboretum	*Macroglossum pyrrhosticta*	77%	*Daphnis nerii*, *Agrius cingulata*, *Helicoverpa zea*	3%
Tantalus	*Macroglossum pyrrhosticta*	67%	*Daphnis nerii*, *Agrius cingulata*, *Helicoverpa zea*	2%
UH Mānoa	*Daphnis nerii, Danaus plexippus*	38%	*Daphnis nerii*, *Helicoverpa zea*	6%
Palehua Palikea	*Hyles calida*	0.00%	*Daphnis nerii*, *Helicoverpa zea*	2%
Total		78%		3%

Parasitoids found

A total of three parasitoid species were reared from wild collected eggs: *Ooencyrtus pallidipes* (Ashmead) (Hymenoptera: Encyrtidae), *Trichogramma chilonis* Perkins (Hymenoptera: Trichogrammatidae), and *Telenomus* sp. indet (Hymenoptera: Scelionidae). Three parasitoids were reared from sentinel egg trials including *O. pallidipes* and *T. chilonis*. No *Telenomus* sp. were recovered in the sentinel trials but an unknown eupelmid was reared six times. All parasitoids were identified by morphology using a key to Families of Hymenoptera by Huber & Goulet (1993). For DNA amplification products, I tried to identify all parasitoids through a BLAST search, but the only parasitoid with a 100% match was *Trichogramma chilonis* for the CO1 gene region. The closest BLAST match to the *Ooencyrtus* vouchers was an undescribed *Ooencyrtus* sp. from the ITS2 region at 96.84%. The closest match to the *Telenomus* species collected was *T. nawai* from the ITS2 region with a match of 85.21%. The unknown eupelmid did not have any close DNA matches from the ITS2 gene region with wasps from both

Pteromalidae and Encyrtidae matching at 85%. The CO1 sequence was not long enough for a successful identification. Sequences were submitted to GenBank; reference numbers can be found with their respective primers in Table 3.3. The eupelmid was only collected 6 times in sentinel sphingid eggs (*D. nerii*) and no similar vouchers were found in the Bishop Museum or the University of Hawai‘i Insect Museum. *T. chilonis* and *O. pallidipes* were purposefully introduced to suppress lepidopteran pests (Funasaki et al., 1988; Mau et al. 1980).

Parasitism rates by host species

Among the lepidopteran species surveyed for parasitoids, the two butterflies *Danaus plexippus* L. (Lepidoptera: Nymphalidae) and *Lampides boeticus* L. (Lepidoptera: Lycaenidae) incurred no parasitism from 17 and 133 collected eggs, respectively, while the sphingids *Daphnis nerii* and *Macroglossum pyrrhosticta* had high levels parasitism, with *M. pyrrhostica* having the highest levels. An egg parasitoid survey of wild caught eggs done on O‘ahu in 2011 found similar results with *D. plexippus* having no parasitism, *D. nerii* with high parasitism and *M. pyrrhosticta* with the greatest (Pacilly et al. 2011). *Macroglossum pyrrhosticta* is an adventive sphingid moth native to Southeast Asia. The larvae feed on the invasive skunk vine: *Paederia foetida* which is native to Asia but found in south-eastern North America and Hawai‘i. The vine is easily found in the Ko‘olau Mountains being is most common at low to mid elevations mesic sites.

Percentage of parasitism by egg species, location, and trial

At sites where I collected data from both wild and sentinel eggs, the overall percentage of parasitism was much greater in wild caught eggs (78%) than in sentinel eggs (3%). Percentage

parasitism was greater in wild caught eggs from every site except for Palehua Palikea. This is likely because only two wild eggs were collected from Palehua Palikea as compared with the 392 sentinel eggs that I retrieved from this site. The locations shared between wild caught and sentinel eggs did not rank the same in order from lowest to highest but were close. Parasitism of sentinel eggs generally increased as elevation decreased, with Upper Mānoa Valley as the exception. In the wild caught eggs, UH Mānoa had the second lowest parasitism next to Palehua Palikea even though UH Mānoa was at the lowest elevation and had a much different (drier) environment and climate.

Wild collected eggs had a negative correlation ($r = -0.72$; $P = 0.167$) between percent parasitism and distance to port from a small sample of localities ($n = 5$) with more parasitism occurring closer to ports. There was also a moderate negative correlation ($r = -0.52$; $P = 0.37$) between percent parasitism and elevation in the wild collected eggs. The sentinel eggs had a small negative correlation ($r = -0.37$; $P = 0.55$) between parasitism and distance to port with the same sample size as wild eggs ($n = 5$), and a moderate negative correlation ($r = -0.54$; $P = 0.35$) between percent parasitism and elevation.

Discussion

The purpose of this study was to assess the species richness and distribution of introduced and native egg parasitoids for Lepidoptera on the island on Oʻahu, across an elevational gradient. In doing so I developed a species list of lepidopteran egg parasitoids in a range of habitats on Oʻahu, attempted to find previously unrecorded species, hosts, and compared parasitism rates and parasitoid species collected from wild eggs versus sentinel egg cards. While no new adventive species were detected, this study suggests that detecting new adventive egg parasitoids

is likely most effectively done with the collection of wild caught eggs and that a range of elevations should be used to survey for new and established species. This result likely holds true for other regions, not just Hawai‘i. By documenting the egg parasitoid species found on various lepidopteran hosts we know which Lepidoptera may be most vulnerable to parasitoids, with possible ramifications for native species in decline. Additionally, this information is useful to inform future biological control projects regarding the current lepidopteran egg parasitoid fauna and their impacts on potential Lepidoptera introductions for weed control.

No unequivocal conclusions can be made in regard to parasitism between wild caught and sentinel eggs because this study was not designed to directly compare parasitism in sentinel and wild caught eggs. The sentinel eggs were deployed on different species of plants than the ones used for harvesting wild eggs, and the species of Lepidoptera between the two studies were not identical, but rather the result of species that were encountered and those that were reared for use as sentinels. However, Jones et al. (2014) compared parasitism between sentinel and wild eggs of the exotic brown marmorated stink bug (Pentatomoidae: *Halymorpha halys*) and found that sentinel eggs had much lower rates of parasitism than wild egg masses. Wright and Diez (2011) measured parasitism in sentinel eggs of the southern green stinkbug (Pentatomidae: *Nezara viridula*) and while this study did not compare parasitism with wild caught eggs, it found low levels of parasitism across various habitats, suggesting these results are part of a broader pattern (Wright and Diez 2011).

Currently there are at least 147 known species of egg parasitoid attacking various arthropods across the Hawaiian islands (See Chapter 1) and a majority of these can be found on O‘ahu: O‘ahu alone has 73 species of Mymaridae (Nishida 2002). The study conducted by Pacilly et al. (2011) collected eggs from at least nine species of lepidoptera (Lycaenidae were all

listed under the family name as “Lycaenid”) at 19 locations, all of which were at or close to sea level, and 11 species of parasitoids were collected. The study by Pacilly et al. found seven more parasitoid species than I did but they also observed less parasitism (38.3%) than I did from a greater number of eggs collected (3881) compared to my 540 which had 77% parasitism. The greater number of sphingid eggs collected in my study might be the cause for the greater rate of parasitism. Most of the eggs collected in Pacilly et al. (2011) were Lycaenidae (N = 2,640) which were only parasitized by various Trichogrammatidae species. The other egg parasitoids, *Telenomus* sp., *Ooencyrtus* sp., *Anastatus* sp., and one unknown Encyrtidae, all came from Sphingidae eggs (N = 422) with *Ooencyrtus* being the most prevalent genus (Pacilly et al. 2011). The non-trichogrammatid wasps were not identified to species in Pacilly et al. but two of the families, Encyrtidae and Scelionidae, were also found in my study. The results of Pacilly et al. (2011) further corroborate that Sphingidae eggs are more suitable for collecting higher numbers of parasitoid species, but it also shows that a greater variety of species of host eggs collected is optimal for finding more species of egg parasitoids.

Parasitism and elevation

Eggs of *M. pyrrhosticta* were not found on UH Mānoa campus, leaving *D. nerii* as the main source of wild caught Sphingidae eggs found there. I believe the lower parasitism rate recorded at UH Mānoa is a direct result of not finding as many sphingid eggs, thus lowering the probability of finding parasitized eggs. Eggs of *M. pyrrhostica* are found in abundance with their host plant due, in part, to the plant’s invasive nature. Finding eggs of *D. nerii* on campus proved to be more difficult because it relies on ornamental plants that get pruned regularly. The same species of parasitoids were collected from *D. nerii* and *M. pyrrhosticta* at locations where

sentinel *D. nerii* and wild *M. pyrrhostica* were collected, and total percent parasitism was very similar between both sphingid species, with 76% parasitism in *D. nerii* from UH Mānoa and 81% total in *M. pyrrhosticta* from all locations pooled. The low level of parasitism from Palehua Palikea is most likely not a direct result of its elevation, but rather a combination of factors such as the proportion of lepidoptera and the number of species available also reflected with the UH locality.

Both wild collected eggs and sentinel eggs showed a moderate negative correlation between percent parasitism and elevation, and it is possible that, with a larger sample size of localities, the correlations could be greater.

Percent parasitism increased as the proximity to ports decreased in wild caught eggs. This might be explained by a greater number of non-native species having been introduced through imported goods and as a result being more abundant in these areas. Sentinel eggs did not show the same strength of correlation, but sentinel eggs were also not a very good indicator for wild parasitism rates in general at a given locality.

Parasitoids found

All parasitoids found in this study were the same species as in the sentinel egg trials (see chapter 2) except for one *Telenomus* sp. This species was not detected using sentinel eggs and was only found at Tantalus, Lyon Arboretum and Upper Mānoa Valley. Voucher specimens, apparently of the same species were found at Bishop Museum, but these were also unidentified. The closest and only two ITS2 BLAST results matching this undescribed *Telenomus* were *T. nawai* at 85% and *T. remus* at 83%. There are currently 6 described *Telenomus* species recorded across the Hawaiian islands and only *T. nawai* is documented to be a lepidopteran egg parasitoid

(Swezey 1929). However, *T. nawai* was only found on eggs from the noctuid *Spodoptera mauritia* (Swezey 1929) and our undescribed species was only collected from sphingid eggs which suggests, along with the DNA, that this is most likely a different species. The eupelmid was only recovered in sentinel eggs which means both sentinel and wild collected eggs retrieved the same number of egg parasitoid species (3). Notably, the *Telenomus* sp. only appeared in wild collected eggs and the eupelmid was only reared from sentinel eggs.

Considering the parasitoids collected, a new host record was observed for *O. pallidipes,* which had previously not been recorded on *D. nerii* nor *A. cingulata*. The encyrtid, *O. pallidipes,* was introduced at Waiāhole and Kāneʻohe on Oʻahu in 1973 for the control of the banana skipper, *Erionota thrax* (Mau et al. 1980). There have been no other studies documenting the localities for the parasitoids collected in this study aside from Pacilly et al. (2011) and the documented release of *O. pallidipes* then listed as *O. erionotae* Ferriere.

Future Studies

Despite significant effort, we were unable to find wild eggs at higher elevations in additional native habitats. Only two eggs were found in a predominantly native forest over the entire duration of this study, neither of which were parasitized, thus, using sentinel eggs might be the only viable way to assess egg parasitoids in this habitat, though any such study should carefully consider the limitations of sentinel assays.

The more effective eggs for collecting egg parasitoids would appear to be Sphingidae as evidenced by their high rates of parasitism in wild caught eggs from *D. nerii* (76%) and *Macroglossum* (81%). Pacilly et al. (2011) also found eggs of Sphingidae to have greater rates of parasitism than those of Noctuidae, Lycaenidae, and Nymphalidae. Eggs of *H. zea* are much

smaller than eggs of *D. nerii* and the only egg parasitoids collected from the latter were *T. chilonis* (See Chapter 2), but this difference could also be due to the noctuid only being used as sentinels and not wild caught eggs. It should be noted that the most thorough way of assaying parasitoids in the field is to collect and or deploy/use eggs from many different species of Lepidoptera in different families based on the results of this study and Pacilly et al. (2011).

It would be interesting in the future to carry out a study purely focused on comparing parasitism in wild collected eggs with those of sentinel eggs; in the current study collecting wild eggs at lower elevations in disturbed forests yielded higher rates of parasitism than using sentinel eggs. The majority of the eggs deployed in the sentinel study were from the sphingid *D. nerii* (N = 1,111) vs the 460 eggs used from *Helicoverpa zea;* the total parasitism from the sentinel sphingid eggs amounted to a mere 4.27% which indicates that the usage of noctuid eggs alone in the sentinel egg study cannot explain the difference in parasitism between wild collected eggs and sentinel eggs. The most effective method for surveying egg parasitoids should be collection of wild eggs if they can be reasonably and easily found in a habitat of interest. If wild eggs are scarce, then sentinel eggs could be used as a secondary method for sampling a habitat. Both wild caught eggs and sentinel eggs detected the same number of species despite the drastically different rates of parasitism, making them both useful for assaying parasitoid presence in a location.

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